CUK-H06042-6-P25464

Volume 93, Number 1, July 1975

British Journal of Dermatology

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Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007-0963

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1. Brit. J. Derm. **92**, 585, 1975
2. Archs. Derm. **86**, 608, 1962
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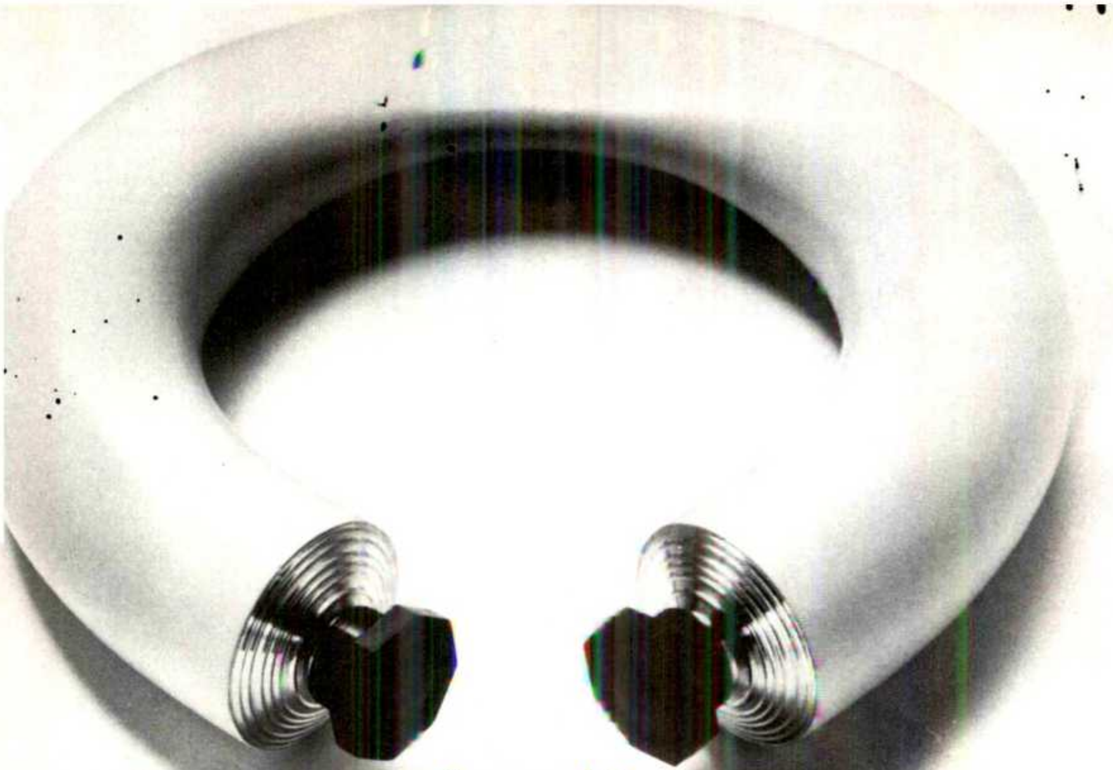**Can one topical steroid combine both fluorinated effectiveness and hydrocortisone safety?**

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2. Wagner D.E., et al., Oncology **26** 310-316 (1971)
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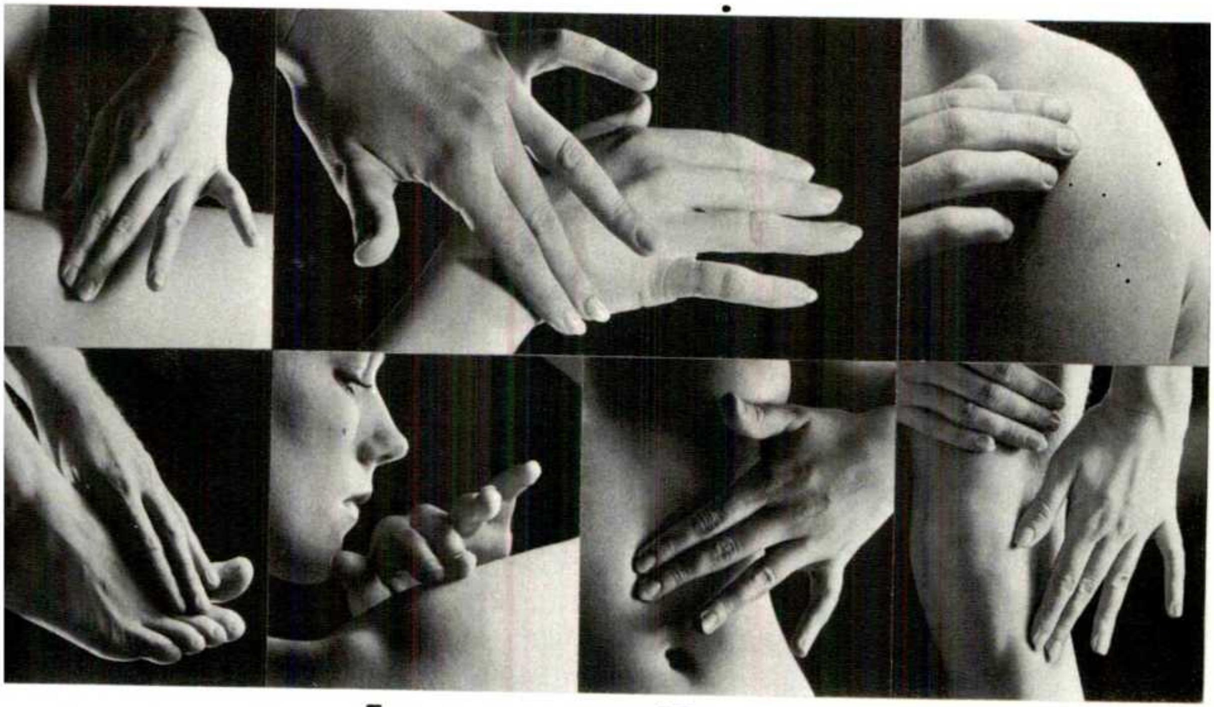

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1. Gaylarde & Sarkany. *Trans. St. Johns Hosp. Derm. Soc.* (1973), **59**, (2), 235–240.
2. Gaylarde & Sarkany. *Trans. St. Johns Hosp. Derm. Soc.* (1973), **59**, (2), 241–243.
3. Woodford & Barry. *Current Therp. Rsch.* 16, **4**, 338–345.
4. Woodford & Barry. *Brit. J. Derm.* (1974), **91**, 323–338.

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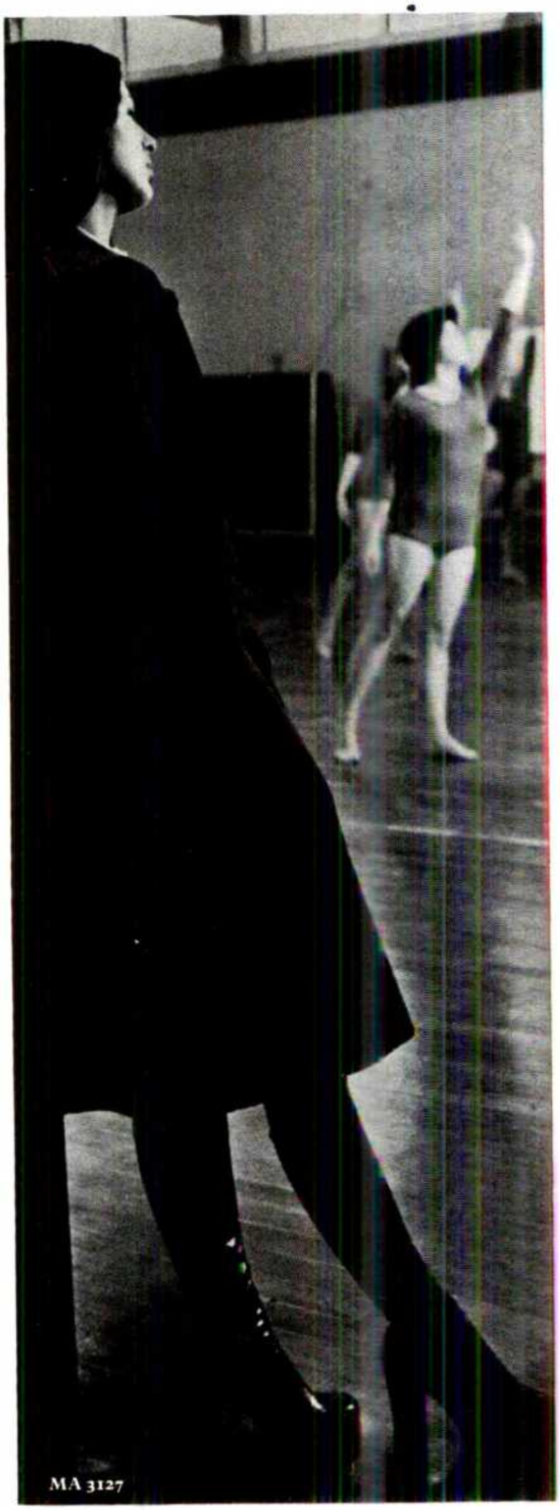

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1. Almeyda, J. and Burt, B.W., *Brit. J. Derm.* (To be published) 2. Harries, E. H. L. and Jacoby, R. H. (To be published)

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*Practitioner, (1974), **213,** 864.

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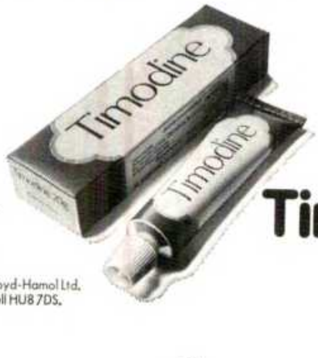

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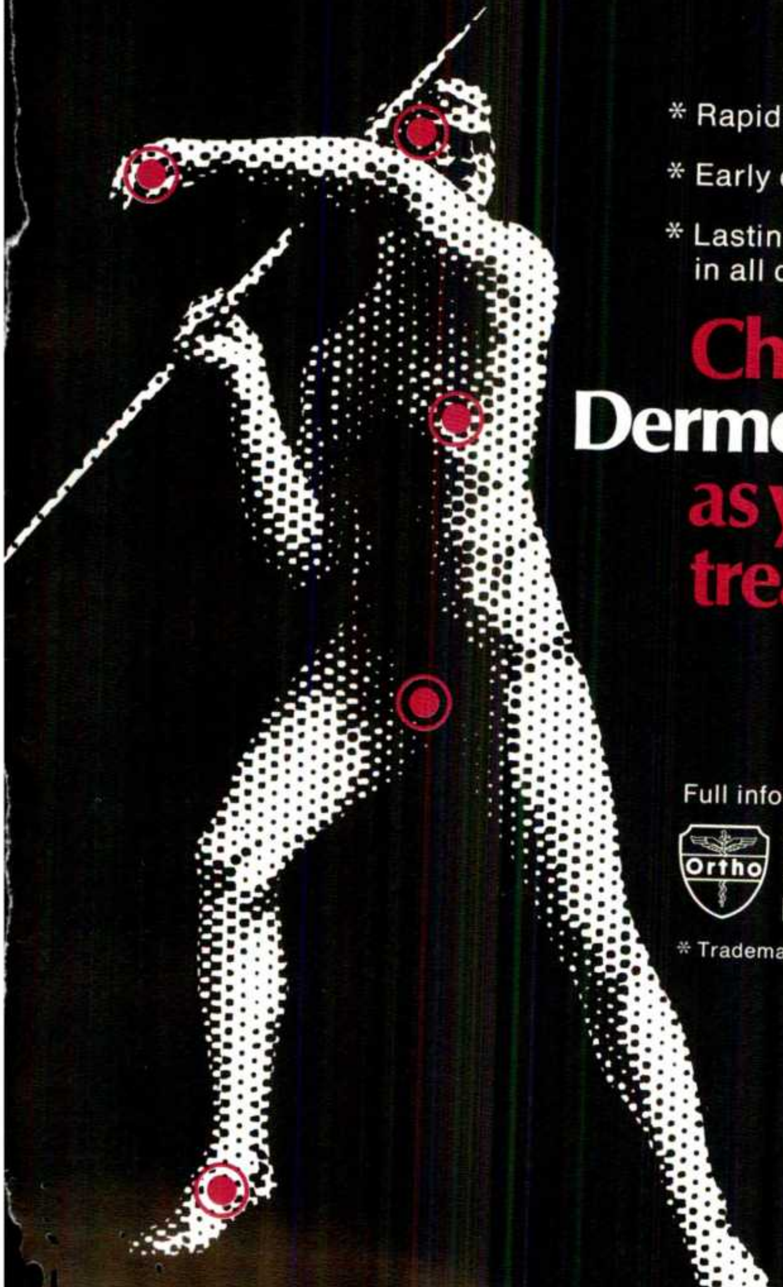

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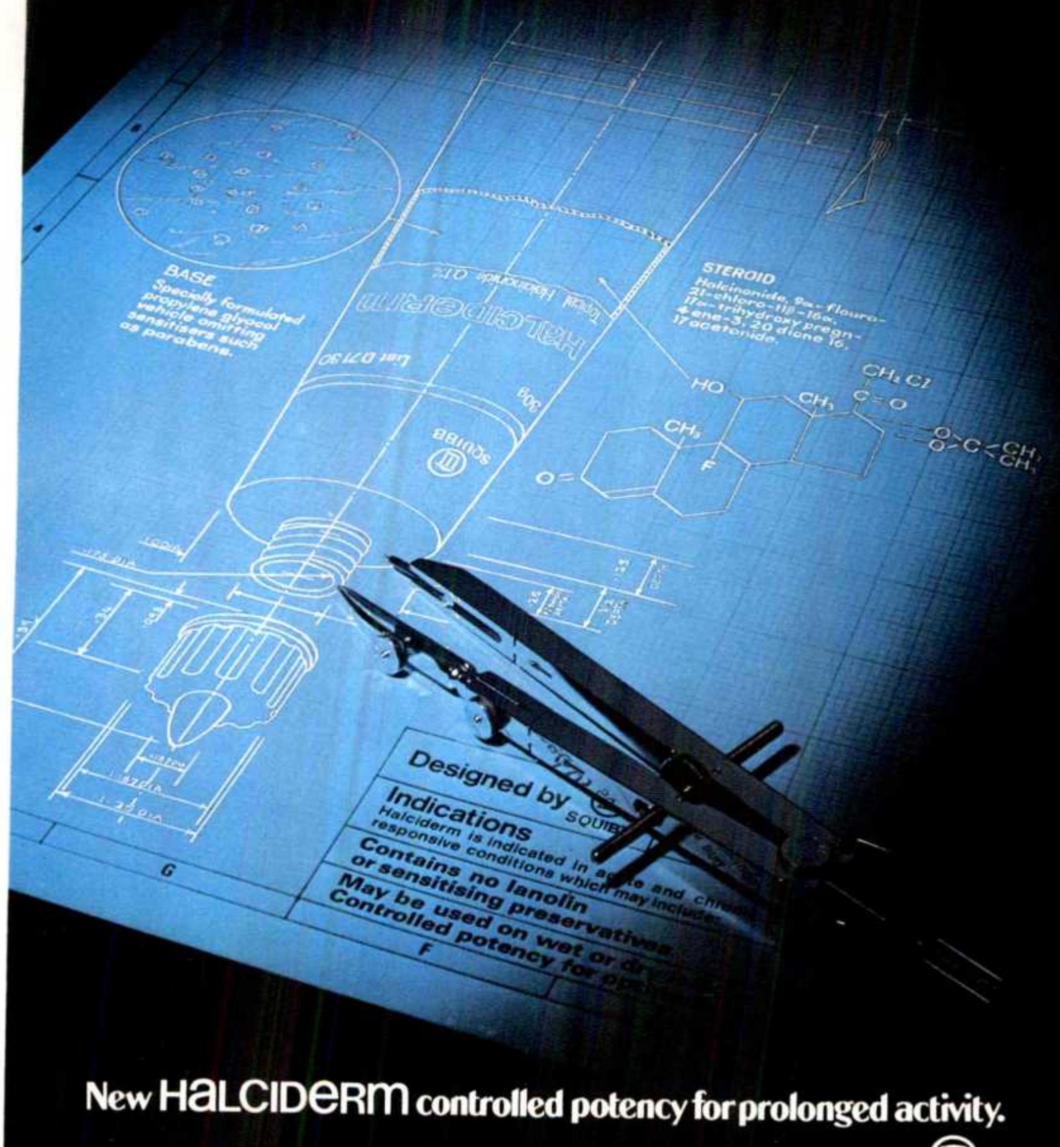
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Index Vol. 149, No. 5, 1974

Barrière, H.; Litoux, P. et Géraut, C. (Nantes): Etude des cellules cornées du psoriasis en microscopie électronique à balayage
(Study of Psoriasic Horny Cells with the Scanning Electron Microscope)

Pierard, G.E.; Hermanns, J.F. et Lapière, C.M. (Liège): Stéréologie de l'interface dermo-épidermique. Observation de la plasticité de la membrane basale au microscope électronique à balayage
(Stereology of the Dermo-Epidermal Junction)

Förster, F.J.; Kallenberg, L.; Fertig, U., and Leonhardt, G. (Frankfurt a.M.): Subcellular Distribution and Cytoplasmatic Kinetic Properties of Acid Phosphatase of Normal Stratum Corneum and Psoriatic Scales

Mier, P.D. and Hurk, J.J.M.A. van den (Nijmegen): Direct Biochemical Assay of the Lipase Activity of Epidermis

Hellgren, L. and Larsson, K. (Gothenburg): On the Effect of Urea on Human Epidermis

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Vermeer, B.J. and Heremans, G.F.P. (Leiden): A Case of Growth Retardation and Cushing's Syndrome Due to Excessive Application of Betamethasone-17-Valerate Ointment

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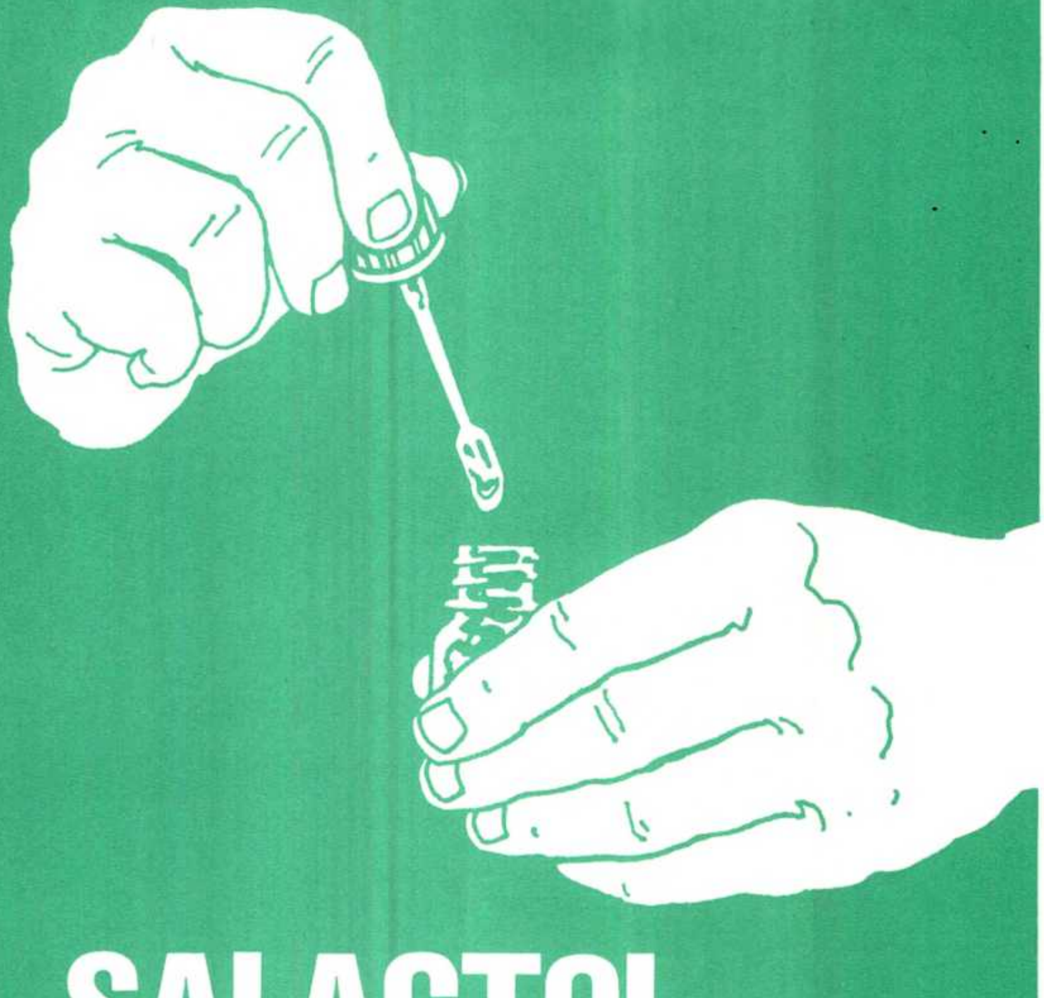

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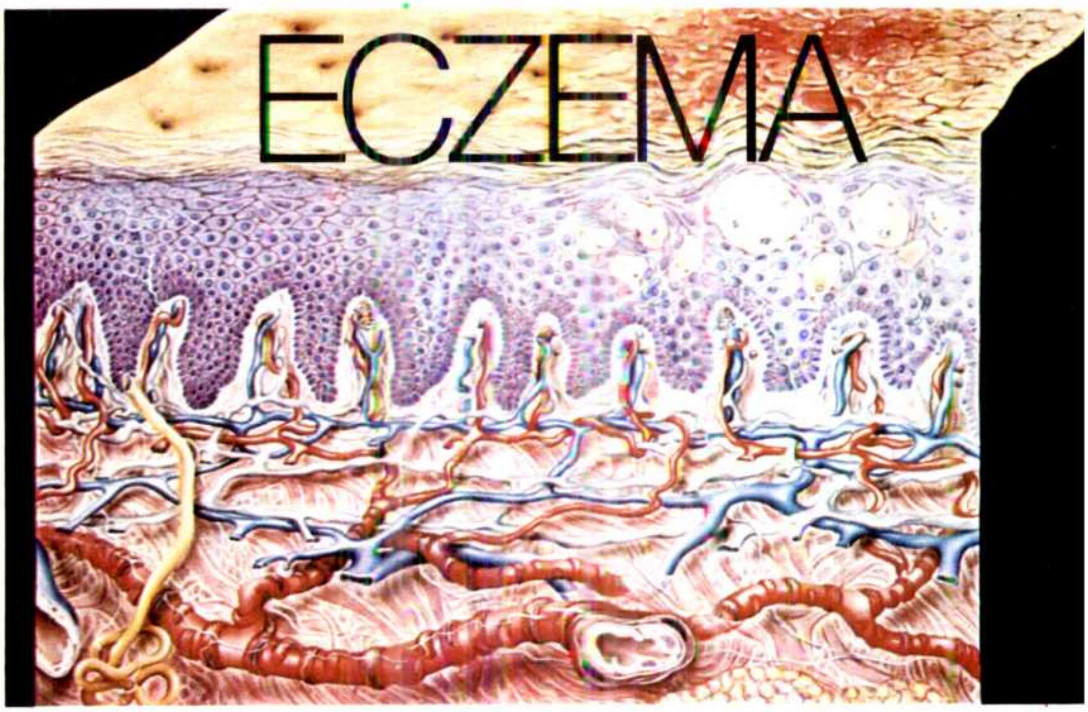

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2) *Wiener Medizinische Wochenschrift*, (1973), **15**, 247-248.
3) *Current Therapeutic Research*, (1972), **14**, 259.

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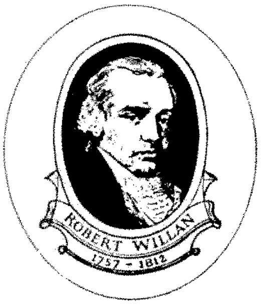

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Contents

No. 1: JULY 1975

Page

Clinical and Laboratory Investigations

Lysosomal Hydrolases of the Epidermis. 1. Glycosidases ... 1
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK
Prealbumin Bearing Lymphocytes as an Indicator of Hapten Protein Carrier in Certain Varieties of Dermatitis ... 11
S. HUSZ, F. HAMERLINCK AND R. H. CORMANE
The Diagnostic Significance of Immunoglobulin and Fibrin Deposition in Lichen Planus ... 17
E. ABELL, D. G. C. PRESBURY, R. MARKS AND D. RAMNARAIN
Deficiency of Circulating IgA and IgM in Adult Patients with Lichen Planus ... 25
L. STANKLER
The Effects of Catecholamine and Related Compounds on the Adenyl Cyclase System in the Epidermis 29
KUNIHIKO YOSHIKAWA, KENJI ADACHI, KENNETH M. HALPRIN AND VICTOR LEVINE
The Effect of Treatment on Plasma Uroporphyrin Levels in Cutaneous Hepatic Porphyria ... 37
B. R. ALLEN, SHEENA PARKER, G. G. THOMPSON, M. R. MOORE, F. J. DARBY AND J. A. A. HUNTER
Repeated Tissue Sampling with a Dental Broach. A Trial in Cutaneous Leishmaniasis ... 43
W. A. D. GRIFFITHS AND W. DUTZ
The Percutaneous Penetration of Nandrolone Decanoate ... 47
M. I. FOREMAN AND I. CLANACHAN
Secondary Syphilis: a Clinico-pathological Review ... 53
E. ABELL, R. MARKS AND E. WILSON JONES
Rosacea of Common Male Baldness ... 63
MALGORZATA GAJEWSKA

Pharmacology and Treatment

Evaluation of Coal Tar Fractions for Use in Psoriasiform Diseases Using the Mouse Tail Test. III. High Boiling Tar Oil Acids ... 67
ROSANNE WRENCH AND ADAM Z. BRITTEN
Evaluation of Dithranol and a 'Synthetic Tar' as Anti-psoriatic Treatments Using the Mouse Tail Test 75
ROSANNE WRENCH AND ADAM Z. BRITTEN
Efficacy of Miconazole in the Topical Treatment of Tinea Pedis in Sportsmen ... 79
J. C. GENTLES, G. R. JONES AND D. T. ROBERTS
Granuloma Annulare and Necrobiosis Lipoidica Treated by Jet Injector ... 85
G. SPARROW AND E. ABELL

Case Reports

Pseudoxanthomatous Mastocytosis ... 91
W. A. D. GRIFFITHS AND K. DANESHBOD
Urticaria and Hyperthyroidism ... 97
J. L. PACE AND MAURICE GARRETTS
Recurrent Necrosis of the Skin and the Mouth ... 101
NABIL I. ELSAHY
Methotrexate Pneumonitis in a Psoriatic ... 107
E. FROM

Comment

The Psoriasis Sufferer in the Community ... 111
R. B. COLES AND T. J. RYAN

Book Review ... 113

Review Article

Immunosuppression and Skin Infection ... 115
J. A. SAVIN AND W. C. NOBLE

No. 2: AUGUST 1975

Page

Clinical and Laboratory Investigations

Naevus Superficialis Lipomatosus: A Clinicopathological Report of Twenty Cases 121
EDWARD WILSON JONES, RONALD MARKS AND D. PONGSEHIRUN

Reactions to Aspirin and Food Additives in Patients with Chronic Urticaria, Including the Physical Urticarias 135
H. M. G. DOEGLAS

One Hundred Twin Pairs Patch Tested with Primary Irritants 145
ROLF HOLST AND HALVOR MÖLLER

Hypersensitive and Insensitive Extremities: The Dermatological Aspects 151
SAMUEL L. MOSCHELLA

Temperature Dependence of Water Content of Stratum Corneum 159
T. S. SPENCER, C. E. LINAMEN, W. A. AKERS AND H. E. JONES

Intraendothelial Tubular Aggregates in Experimental Wounds: Ultrastructural Study 165
R. A. J. EADY AND G. F. ODLAND

Long-spacing Collagen in Skin Biopsies from Patients with Lepromatous Leprosy 175
ROSA P. EDWARDS

Hazards of Steroid Therapy in Hepatic Failure 183
W. J. CUNLIFFE, J. L. BURTON, G. HOLTI AND V. WRIGHT

Tissue Nickel Levels and Nickel Dermatitis. I. Nickel in Hair 187
S. A. KATZ, H. J. M. BOWEN, J. S. COMAISH AND M. H. SAMITZ

Physiological and Environmental Control of Gram Negative Bacteria on Skin 191
M. E. MCBRIDE, W. C. DUNCAN AND J. M. KNOX

Pruritus Vulvae: Treatment by Multiple Intradermal Alcohol Injections 201
G. D. WARD AND J. R. SUTHERST

Pharmacology and Treatment

Simplified Dithranol Treatment for Psoriasis 205
R. H. SEVILLE

The Absorption of Hexachlorophane from Ultralanum Ointment 209
PATRICK G. T. BYE, WARWICK MORISON AND ELLEN L. RHODES

Case Reports

Generalized Bullous and Haemorrhagic Lichen Sclerosus et Atrophicus: Marked Improvement with ACTH 215
ADRIANO DI SILVERIO AND FERDINANDO SERRI

Reactivation of Scabies Rash by Methotrexate 219
D. BURROWS, J. M. BRIDGES AND T. C. M. MORRIS

Brief Communications

Fourier Analysis of Thermal Sweating 223
D. W. K. COTTON, G. J. DE JONGH AND F. A. J. THIELE

Impulse Cytophotometry in Psoriasis 225
F. W. BAUER AND R. M. DE GROOD

Vitiligo and Jaundice 229
H. NEERING

Comment

The Choice and Use of Equipment for Photomicrographs 231
J. A. MILNE

Book Reviews 235

News and Notices 238

No. 3: SEPTEMBER 1975

Page

Clinical and Laboratory Investigations

Intra-epidermal Carcinoma of the Eyelid Margin 239
D. I. MCCALLUM, P. D. C. KINMONT, D. WYNN WILLIAMS, E. E. COTTON AND M. A. WROUGHTON

Is the Cylic AMP in Psoriatic Epidermis Low? 253
K. YOSHIKAWA, K. ADACHI, K. M. HALPRIN AND V. LEVINE
Survey of Skin Disorders in the Urban Black Population of South Africa 259
M. DOGLIOTTI
A Method of Liberating Living Cells from the Dermal Infiltrate: Studies on Skin Reticuloses and Lichen Planus 271
R. S-H. TAN, N. A. BYROM AND J. P. HAYES
Thin Layer Chromatography and Counter-current Analysis in Porphyrias 277
J. PIÑOL AGUADÉ, C. HERRERO, J. ALMEIDA, S. G. SMITH AND R. V. BELCHER
The Use of Thin Layer Chromatography in the Separation of Free Porphyrins and Porphyrin Methyl Esters 291
S. G. SMITH
Further Studies of Scratching During Sleep 297
J. A. SAVIN, W. D. PATERSON, I. OSWALD AND KIRSTINE ADAM
A New Method for the Measurement of Itch and the Response to Treatment 303
R. FELIX AND SAM SHUSTER
Coexistence of Lichen Planus and Bullous Pemphigoid: an Immunopathological Study 313
G. STINGL AND K. HOLUBAR
A Prospective Study of the Effects of Weekly Oral Methotrexate on Liver Biopsy: Findings in Severe Psoriasis 321
A. P. WARIN, J. W. LANDELLS, G. M. LEVENE AND H. BAKER

Case Reports

Cowden Disease: Report of Two Additional Cases 329
JOSEPH W. BURNETT, RONALD GOLDNER AND GARY J. CALTON
Deafness Complicating Mucous Membrane Pemphigoid: a Case Report 337
JOHN THOMSON, WILLIAM LANG AND J. A. CRAIG
Unusual Cutaneous Associations of a Malignant Carcinoid Tumour of the Bronchus—Erythema Annulare Centrifugum and White Banding of the Toe Nails 341
J. D. EVERALL, P. M. DOWD AND B. ARDALAN

Brief Communications

Sebum Excretion Rate Investigations: a New Absorbent Paper 347
W. J. CUNLIFFE, S. M. WILLIAMS AND S. G. TAN
Electron Microscopy of the Sweat Glands from the Hairy Skin of the Beagle 348
D. W. K. COTTON, P. VAN HASSELT AND A. M. S. BERGERS
Hair Fragment in the Skin resembling Larva Migrans 349
EERO A. LEHMUSKALLIO

Comment

The Safety of Fragrance Ingredients 351
DONALD L. OPDYKE

Correspondence

Erythrocyte Glucose-6-phosphate Dehydrogenase in Lichen Planus 353
W. K. JACYK, D. W. K. COTTON AND W. B. J. M. VAN DER STAAK
Book Reviews 355

No. 4: OCTOBER 1975

Page

Clinical and Laboratory Investigations

Pigmented Basal Cell Epithelioma: Light and Electron Microscopic Studies on Tumours and Cell Cultures 361
STANLEY S. BLEEHEN
Pigmentary Demarcation Lines: Comparison of Negroes with Japanese 371
VICTOR J. SELMANOWITZ AND JAMES M. KRIVO
Absorption and Biotransformation of Topically Applied 8-(methylthio)cyclic AMP: Studies in Dogs and Humans 379
JACQUES DREYFUSS, TERRENCE W. MISCHLER, EDWARD S. NEISS, JAMES M. SHAW AND ERIC C. SCHREIBER
Lysosomal Hydrolases of the Epidermis. 2. Ester Hydrolases 391
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK

Median Rhomboid Glossitis: Candidiasis and not a Developmental Anomaly ... 399
BRIAN E. D. COOKE
Plane Xanthomata Associated with Multiple Myeloma and Hyperlipoproteinaemia ... 407
K. J. MARIEN AND G. SMEENK
Dermo-epidermal Adhesion and its Effect on Epidermal Structure in the Mouse ... 417
WILLIAM S. BULLOUGH AND JOHANNA U. R. DEOL
The Differential Binding of Antibody from the Sera of Patients with Pemphigus and Pemphigoid to Isolated Guinea-pig Epidermal Cells ... 425
W. D. BRIGDEN AND H. E. AMOS
Immunohistological Studies, with Anti-connective Tissue and Anti-immunoglobulin Antisera, of the Skin in Lupus Erythematosus and Scleroderma ... 431
N. R. ROWELL AND D. G. SCOTT
The Deoxyribonucleic Acid (DNA) Skin Test in Systemic Lupus Erythematosus. 1. Clinical Evaluation 443
E. A. JOHANSSON, U. KIISTALA, K-M. NIEMI, H. HALME AND A. LASSUS
The Deoxyribonucleic Acid (DNA) Skin Test in Systemic Lupus Erythematosus. 2. Histological Findings 451
E. A. JOHANSSON, K-M. NIEMI AND A. LASSUS
An Improved Method of Assessing Topical Corticosteroid Activity ... 459
H. BARNES, P. M. GAYLARDE, A. P. BROCK AND I. SARKANY

Case Report

Lichen Planus and Ulcerative Colitis ... 465
EDWARD H. WYATT

Brief Communications

Membrane-bound ATP-hydrolytic Activity of Psoriatic Epidermis ... 469
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK
Inhibition of Lysosomal Enzymes by Dapsone ... 471
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK

Correspondence

Induced Tolerance in Physical Urticarias ... 473
J. S. PEGUM
Cutaneous Protothecosis ... 473
M. DOGLIOTTI, P. W. MARS, A. R. RABSON AND J. J. RIPPEY

Book Reviews ... 474

Review

Dispersal of Skin Microorganisms ... 477
W. C. NOBLE

News and Notices ... 486

No. 5: NOVEMBER 1975

Page

Clinical and Laboratory Investigations

Natural Antibiosis Among Skin Bacteria as a Primary Defense Against Infection ... 487
SYDNEY SELWYN
Ultrastructural Localization and Differentiation of Membrane-bound ATP Utilizing Enzymes Including Adenyl Cyclase in Normal and Psoriatic Epidermis ... 495
G. MAHRLE AND C. E. ORFANOS
Lysosomal Hydrolases of the Epidermis. 3. Peptide Hydrolases ... 509
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK
Livedo Reticularis. Signs in the Skin of Disturbance of Blood Viscosity and of Blood Flow ... 519
P. W. M. COPEMAN
The Significance of a Positive Cutaneous Immunofluorescence Test in Systemic Lupus Erythematosus ... 531
PAUL I. DANTZIG, JACQUELINE MAURO, SYRUS RAYHANZADEH AND ULRICH H. RUDOFSKY
Cellular Immunity and Circulating Antibody to Herpes Simplex Virus in Subjects with Recurrent Herpes Simplex Lesions and Controls as Measured by the Mixed Leukocyte Migration Inhibition Test and Complement fixation ... 539
R. W. GANGE, A. DE BATS, J. R. PARK, C. M. P. BRADSTREET AND E. L. RHODES

In Vitro Assay of Immunity to Human Wart Antigen 545
WARWICK L. MORISON
Cell-mediated Immune Responses in Patients with Warts 553
WARWICK L. MORISON
Nickel Hypersensitivity. Nickel Binding to Amino Acids and Lymphocytes 557
F. HUTCHINSON, T. M. MACLEOD AND E. J. RAFFLE

Pharmacology and Treatment

Comparative Bio-availability and Activity of Proprietary Topical Corticosteroid Preparations: Vasoconstrictor Assays on Thirty-one Ointments 563
B. W. BARRY AND R. WOODFORD
Treatment of Pemphigus with Cyclophosphamide 573
J. S. PASRICHA, V. D. SOOD AND Y. MINOCHA
A Follow-up of Tetracycline-treated Rosacea with Special Reference to Rosacea Keratitis 577
ARTHUR G. KNIGHT AND C. F. H. VICKERS

Case Reports

Hodgkin's Disease in a Patient with Follicular and Dermal Mucinosis and Spontaneous Ooze of Mucin (Mucinorrhoea) 581
L. STANKLER AND S. W. B. EWEN
Leprechaunism. Case Report in a Black African Child 587
R. G. HARTDEGEN, M. DOGLIOTTI, L. RABINOWITZ AND R. G. BARTLETT
Multiple Keratoacanthomas: Association with Deficient Cell Mediated Immunity 593
A. CLAUDY AND J. THIVOLET

Comment

Classification and Differentiation of Cutaneous Lymphomas. Enzyme-cytochemical and Immunocytological Studies 597
GÜNTER BURG AND OTTO BRAUN-FALCO

Obituaries 601
THERESA KINDLER AND ERNST SKLARZ

Report

Report of the Meeting of the International Committee of Dermatology in Mexico City, 31 October to 3 November 1974 603

Correspondence

Erythropoietic Protoporphyria: Submicroscopic Events During the Acute Photosensitivity Flare 605
T. J. RYAN
Alopecia Areata 605
J. W. H. MALI

Book Reviews 606

News and Notices 611

No. 6: DECEMBER 1975

Page

Clinical and Laboratory Investigations

Exogenous Ochronosis and Pigmented Colloid Milium from Hydroquinone Bleaching Creams 613
G. H. FINDLAY, J. G. L. MORRISON AND I. W. SIMSON
Ultrastructural Study of Elaunin Fibres in the Secretory Coil of Human Eccrine Sweat Glands 623
G. COTTA-PEREIRA, F. GUERRA RODRIGO AND S. BITTENCOURT-SAMPAIO
The Fine Structure of the Elastic Tendons in the Human *Arrector Pili* Muscle 631
F. GUERRA RODRIGO, G. COTTA-PEREIRA AND J. F. DAVID-FERREIRA
The Influence of Age and Sex on Skin Thickness, Skin Collagen and Density 639
SAM SHUSTER, MARTIN M. BLACK AND EVA MCVITIE

Palmar Ridge Appearances in Normal Newborn Infants and Ridge Appearance in Relation to Eccrine Sweating 645
JULIAN VERBOV
Epidermal Transit Times 649
CHRISTOPHER S. POTTEN
Immunological Responses of Patients with Psoriasis and the Effect of Treatment with Methotrexate 659
A. LEVANTINE AND JONATHAN BROSTOFF
Studies on the Energy Metabolism in Lichen Planus 667
HANS HAMMAR
The Lichen Planus-like Eruption after Bone Marrow Transplantation 675
J. H. SAURAT, ELIANE GLUCKMAN, ANETTE BUSSEL, LILIANE DIDIERJEAN AND A. PUISSANT
Arterial Thrombosis in Scleroderma 683
NANCY L. FUREY, F. R. SCHMID, H. C. KWAAN AND H. H. R. FRIEDERICI

Case Reports

Human Infestation by *Ophionyssus Natricis* Snake Mite 695
HANNE SCHULTZ
Keratoacanthoma in a Bantu Patient 699
T. HEYL AND J. G. L. MORRISON
Angioma Serpiginosum—Familial Incidence 701
PHILIP J. MARRIOTT, DOWLING D. MUNRO AND TERENCE RYAN
Hypertrichosis due to Diazoxide 707
J. L. BURTON, W. H. SCHUTT AND I. W. CALDWELL
Acquired Pigmentation of Skin Folds with the Histological Picture of Seborrhoeic Wart 713
C. A. KAMINSKY, A. R. DE KAMINSKY, O. SANGUINETTI AND M. SHAW

Brief Communications

Wart Immunity, Autoantibodies and Australia Antigen in Sarcoidosis 717
WARWICK L. MORISON
Pigmentation of the Palms and Scalp Probably due to Proprietary Hair Tonics, Containing Various Phenols and Phenolic Derivatives 718
L. FORMAN
Erythema Nodosum from *Yersinia Enterocolitica* 719
N. E. BALDOCK AND M. D. CATTERALL

Comment

β-Carotene and the Photodermatoses 721
NORMAN POLLITT

Obituaries

DR W. N. GOLDSMITH 725
DR F. GLYN-HUGHES 728

Society Proceedings

Irish Dermatological Society 729

Correspondence

High Doses of Ascorbic Acid 731
S. G. TAN AND W. J. CUNLIFFE

News and Notices 732

Index to Volume 93 733

British Journal of Dermatology (1975) **93**, 1.

Clinical and Laboratory Investigations

Lysosomal hydrolases of the epidermis

1. GLYCOSIDASES

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Accepted for publication 11 October 1974

SUMMARY

Seven distinct glycosidases (EC 3.2) have been characterized in guinea-pig epidermis. Their properties indicate them to be of lysosomal origin. The 'profile' of the epidermal glycosidases is significantly different from that reported for whole skin, the activities of β-galactosidase and β-acetylglucosaminidase being very high and those of the remaining enzymes relatively low in epidermis.

Lysosomes contain a wide range of hydrolytic enzymes which are unusual in possessing markedly acid pH optima. Because of the growing interest in the lysosomal storage diseases, the distribution of these enzymes has been extensively investigated in a number of different tissues. Several authors have published useful general reviews of the lysosomal enzymes; one of the most comprehensive is that of Barrett (1972).

A number of workers have measured certain of the hydrolases from whole human skin, mainly with a view to assessing the diagnostic value of skin biopsy in the mucopolysaccharidoses. Other workers, with the same objective, have studied cultured fibroblasts derived from skin. Surprisingly, however, our knowledge of epidermal hydrolases is limited almost exclusively to histochemical observations. Quantitative biochemical data are urgently needed, both to clarify the role of lysosomes in normal keratinization and also to evaluate possible changes in disease. A programme for the identification, characterization and quantification of epidermal acid hydrolases has therefore been undertaken in this laboratory.

Lysosomal enzymes may be divided into three main subgroups:

EC 3.1 (ester hydrolases). This group includes various phosphatases and sulphatases, in addition to the carboxylic ester hydrolases.

EC 3.2 (glycoside hydrolases). These attack a wide range of glycosyl compounds, ranging from simple glycosidases to biopolymers such as hyaluronic acid.

EC 3.4 (peptide hydrolases). These include the well-known 'cathepsins'. Precise classification of this group is difficult because of the relatively low specificity of many of the peptidases and proteases.

In the present paper we wish to describe certain of the glycoside hydrolases (EC 3.2) of guinea-pig epidermis. The ester hydrolases (EC 3.1) and the peptide hydrolases (EC 3.4) will be dealt with in subsequent communications.

MATERIALS

Chemicals were in general of analytical grade. Fluorogenic substrates were purchased from either the Sigma Chemical Co. or from Koch-Light Laboratories, Ltd. (Table 1). Bovine serum albumin (BSA) was purchased from Organon B.V. (Netherlands) under the trade name of 'Povite'. De-ionized water was used throughout.

TABLE 1. Substrates and pH values used for the assay of the individual glycoside hydrolases

EC number	Enzyme	Substrate	Source	pH of assay
3.2.1.20	α-glucosidase	4-MU-α-D-glucoside	Sigma	5·5
3.2.1.21	β-glucosidase	4-MU-β-D-glucoside	Sigma	4·6
3.2.1.22	α-galactosidase	4-MU-α-D-galactoside	Koch-Light	3·6
3.2.1.23	β-galactosidase	4-MU-β-D-galactoside	Sigma	3·6
3.2.1.24	α-mannosidase	4-MU-α-D-mannoside	Koch-Light	5·9
3.2.1.30	β-acetylglucosaminidase	4-MU-N-acetyl-β-D-glucosaminide	Sigma	4·3
3.2.1.—	β-acetylgalactosaminidase	4-MU-N-acetyl-β-D-galactosaminide	Sigma	4·3
3.2.1.31	β-glucuronidase	4-MU-β-D-glucuronide	Sigma	3·0

Albino guinea-pigs (6–8 weeks old) were killed by a blow to the head, shaved, and epidermis removed from the back and flanks using a Castroviejo keratotome (Stortz Instrument Co., St Louis, U.S.A.) set for a thickness of 0·4 mm. The epidermis was homogenized in an all-glass homogenizer ('Micro-wet grinder', Townson and Mercer, Croydon) in an ice-cold aqueous solution of 1 mg/ml BSA at a concentration of 20 mg epidermis per ml BSA solution. After a brief centrifugation to remove the larger cell debris, the supernatant was distributed into 1 ml aliquots and stored at −20°C.

All fluorescence measurements were made using a 'Fluorispec' spectrophotofluorimeter (Baird Atomic).

METHODS

Assay procedure

Preliminary experiments suggested that the use of 4-methylumbelliferyl glycosides (4-MU glycosides) offered considerable advantages in sensitivity and convenience over alternative methods currently available. The general technique using these compounds is described below; individual enzymes assayed, together with details of substrate and pH, are listed in Table 1.

For routine assay, the appropriate glycoside was dissolved at a concentration of 1 mmol/l in 0·04 mol/l lactate buffer. If there was appreciable contamination with free 4-MU (indicated by alkaline fluorescence), the solution was extracted twice with an equal volume of ether and the excess ether removed by passing a current of air through the water solution. The buffered substrate solutions were stable at −20°C for several weeks.

The reaction was initiated by the addition of 0·1 ml of epidermal extract (usually diluted to 5 mg/ml with BSA solution) to 0·1 ml of buffered substrate. After incubation for 60 min at 37°C the reaction was stopped by the addition of 2·5 ml of carbonate–bicarbonate buffer (0·2 mol/l, pH 10·5) and the fluorescence measured ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 450$ nm). A blank, in which the epidermal extract was replaced by BSA solution, was incubated with each assay. A standard solution of 4-MU in carbonate-bicarbonate buffer (10^{-6} mol/l) was used for calibration.

After subtraction of the blank, enzymatic activity was calculated as nmoles (10^{-9} mol) of 4-MU liberated per min per g fresh weight of epidermis.

Progress curves

Each of the buffered substrate solutions (1·0 ml) was incubated with 1·0 ml of epidermal extract (5 mg/ml) and a corresponding blank set up using BSA. Aliquots of 0·2 ml were removed from each for fluorimetry (as described above) at intervals up to 2 h. After subtraction of the blank, the net release of 4-MU was plotted as a function of time.

Effect of epidermal concentration

The epidermal extract was diluted with BSA in the range 1–10 mg/ml, and assays were conducted exactly as described above.

Effect of pH

A series of buffers (lactate, 0·04 mol/l) were prepared in the range pH 2·4–5·9. Substrates (1 mmol/l) were dissolved in water. Incubation mixtures contained 0·1 ml of the appropriate substrate, 0·1 ml buffer and 0·1 ml epidermal extract (10 mg/ml); after incubation for 60 min at 37°C, the reaction was stopped and fluorescence measured as described previously.

Duplicate samples and a blank were assayed at each pH value; after subtraction of the blank, the net enzymatic activity was plotted as a function of pH.

Enzyme kinetics

The activity of each enzyme was determined using a series of at least five different substrate concentrations, all other conditions being as described above. Duplicate assays and a blank were measured at each substrate concentration.

The reciprocal of the reaction velocity was plotted as a function of the reciprocal of the substrate concentration (Lineweaver and Burk plot), and the Michaelis–Menton constant (Km) obtained by extrapolation.

Normal values and enzyme stability

The activity of each enzyme was determined in fresh epidermal extracts prepared from four different animals. Measurements were repeated at intervals of up to 2 months during storage of one extract at −20°C.

RESULTS

General

Seven of the eight glycosides (Table 1) were hydrolysed at significant rates. The remaining substrate, 4-MU-N-acetyl-β-D-galactosaminide, was hydrolysed at about 15% of the rate of the corresponding glucosaminide by each extract tested. Since this may be anticipated in the presence of β-acetylglucosaminidase (Barrett, 1972), it was assumed that the extracts contained no specific β-acetylgalactosaminidase. This latter enzyme was not investigated further.

Progress curves

These are illustrated in Figs. 1a and b. It can be seen that all enzymes showed a linear reaction rate

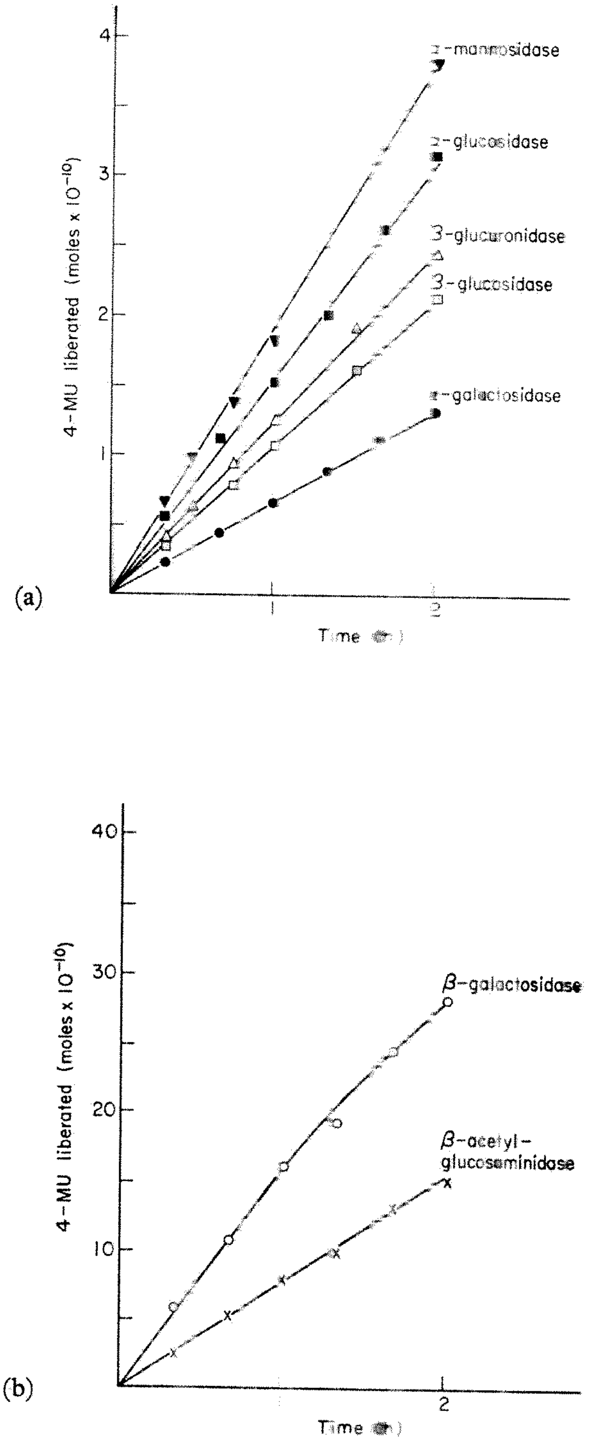

FIGURE 1. Progress curves showing the release of 4-MU from the various glycosides by epidermal extracts for periods up to 2 h.

for at least 2 h with the exception of β-galactosidase. In the case of this enzyme a slight but definite loss of activity was observed after the 1st hour.

Effect of epidermal concentration

The reaction rate was proportional to the concentration of epidermis in the range 1–10 mg/ml for each of the seven enzymes investigated.

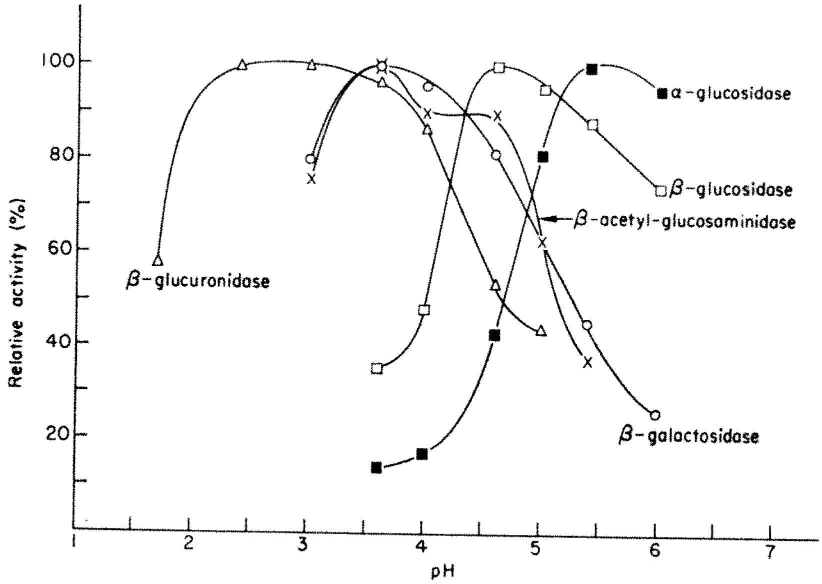

FIGURE 2. The effects of pH on the activities of five of the epidermal glycosidases.

Effect of pH

Five enzymes displayed clear optima in the range examined; the curves are illustrated in Fig. 2 and the pH values listed in Table 2. In the case of α-galactosidase a broad plateau was observed between pH values of 3·0 and 4·6, and in the case of α-mannosidase the optimum lay above pH 5·9.

TABLE 2. The pH optima of epidermal glycosidases (present study) compared with values reported for whole skin and for isolated lysosomal preparations from various sources

		Homogenates of whole skin			
Enzyme	Epidermal extracts (present study)	Steigerwald & Bartholomew (1973)	Goldberg *et al.* (1971)	Öckerman (1969)	Isolated lysosomal preparations (summarized by Barrett, 1972)
α-glucosidase	5·5				3·7–4·5
β-glucosidase	4·6		3·6–5·6	5·0	5·0–5·5
α-galactosidase	3·0–4·6				5·2
β-galactosidase	3·6	4·0	4·6–5·0	4·4	3·0–6·0
α-mannosidase	>5·9			5·4	5·0
β-acetylglucosaminidase	3·6		3·2 and 6·0	5·0	4·5
β-glucuronidase	2·4–3·6	4·0			4·3

Enzyme Kinetics

The Lineweaver and Burk plots for six of the enzymes were linear over the range of substrate con-

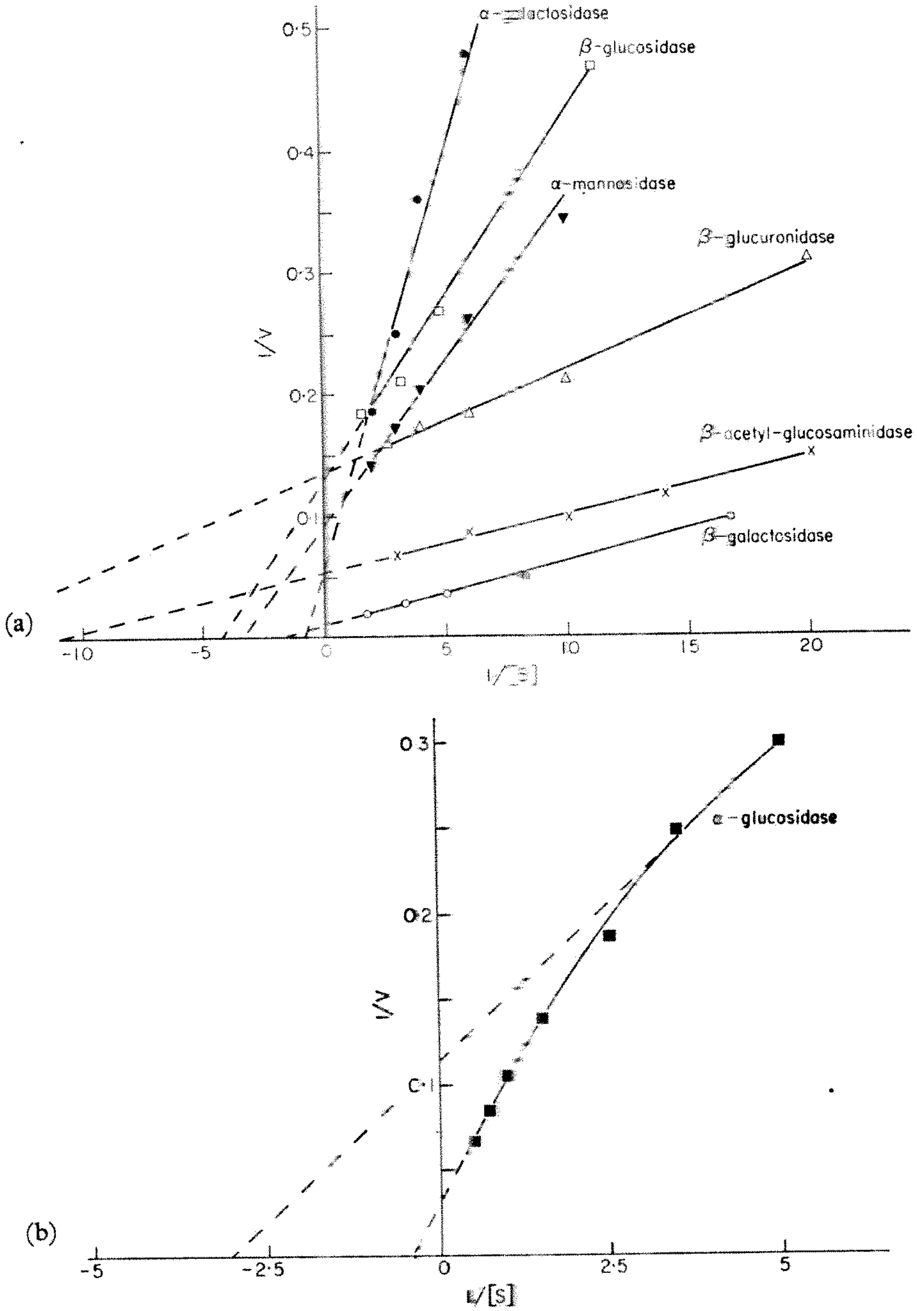

FIGURE 3. Lineweaver and Burk plots showing the effects of substrate concentration on the activities of the epidermal glycosidases. Reaction velocities are in nmol/min/g and substrate concentrations are mmol/l.

centrations employed (Fig. 3a). The remaining enzyme, α-glucosidase, showed a distinctly biphasic curve (Fig. 3b). Michaelis–Menton constants obtained by extrapolation are listed in Table 3.

Normal values and enzyme stability

The activities of each enzyme determined in fresh extracts prepared from four different animals are shown in Table 4.

TABLE 3. Michaelis–Menton constants of epidermal glycosidases for the 4-MU glycosides (present study) compared with values reported for other mammalian tissues. All values are in mmol/l

Enzyme	Epidermal extracts (present study)	Cultured fibroblasts (Hultberg *et al.*, 1973)	Cultured fibroblasts (Kanfer & Spielvogel, 1972)	Nervous tissue (monkey)	Nervous tissue (rat)	Testis (Leaback & Walker, 1961)
				(Robins *et al.*, 1968)		
α-glucosidase	2·5 and 0·033					
β-glucosidase	0·23	0·50		0·067	0·092	
α-galactosidse	1·0					
β-galactosidase	0·62	3·35	0·41 and 0·05	0·108	0·152	
α-mannosidase	0·29	1·78				
β-acetylglucosaminidase	0·094					1·22
β-glucuronidase	0·063			0·058	0·014	

TABLE 4. Activities of epidermal glycosidases in preparations from four different guinea-pigs. All values are in nmol (10^{-9} mol) of 4-MU liberated per min per g fresh weight of epidermis

	Animal no.				
Enzyme	1	2	3	4	Mean
α-glucosidase	4·9	4·1	4·2	4·5	4·4
β-glucosidase	2·5	2·9	1·4	2·4	2·3
α-galactosidase	4·8	5·7	1·8	3·9	4·1
β-galactosidase	36	36	28	37	34
α-mannosidase	2·7	4·3	3·3	9·0	4·8
β-acetylglucosaminidase	20	24	17	38	25
β-glucuronidase	2·4	2·4	2·0	6·0	3·2

In general the enzymes were remarkably stable during storage. The exceptions were β-galactosidase and α-mannosidase, whose activities fell to 10% and 30% respectively of their original levels after 2 months at −20°C.

DISCUSSION

The ultimate sensitivity of a fluorimetric assay is, unlike that of colorimetric methods, limited mainly by the magnitude of the blank. In the technique described here the total 'blank' (including the fluores-

TABLE 5. Values reported for glycosidase activities in whole human skin

Author and year	Öckerman (1969)		Gerich (1969)	Goldberg *et al.* (1971)		Clausen *et al.* (1972)	Steigerwald & Bartholomew (1973)
Substrate	Various		p-nitrophenyl derivatives	4-MU derivatives		p-nitrophenyl derivatives	p-nitrophenyl derivatives
Units Enzyme	μmol/min/ g protein	μmol/min/ g wet wt.	μmol/min/ g protein	μmol/h/ g wet wt.	μmol/min/ g protein	μmol/min/ g protein	μmol/min/ g soluble protein
α-glucosidase		0·0693					
β-glucosidase		0·0168		0·31	0·122		
α-galactosidase							
β-galactosidase	0·390		0·279	1·36	0·539	0·48	3·8
α-mannosidase		0·125		1·64		0·14	
β-acetylglucos-aminidase	2·65		1·71	2·29	0·908		
β-glucuronidase	0·178			1·11	0·440		5·9

cence of the glycoside itself, contamination with free 4-MU, non-enzymatic decomposition of the substrate during incubation and occasional traces of enzyme activity in the BSA) was usually equivalent to less than 20 pmol (2×10^{-11} mol) of 4-MU. Assuming the minimum acceptable assay reading to be double this value, it can be seen that the enzymes described could be measured in amounts of epidermis ranging between 10 and 200 μg; reduction of the reaction volumes (e.g. to 20 μl) would reduce these figures to 1–20 μg. Thus this type of assay is admirably suited either to the determination of enzyme 'profiles' in biopsy material or to the study of microdissected tissue fragments.

Our results show beyond doubt that there are at least 7 distinct glycoside hydrolases present in epidermis. Their relative activities in guinea-pig epidermis are shown in Table 4; these may be compared with values reported previously for whole human skin (Table 5). Despite the usual problems resulting from the use of different substrates, assay conditions and choices of reference variables, it seems evident that the glycosidase profile of epidermis is significantly different from that of whole skin; in particular the activities of β-galactosidase and β-acetylglucosaminidase are relatively much higher in epidermis. The only report dealing with isolated human epidermis (Gibbs & Griffin, 1970) suggested that the β-glucuronidase level is about tenfold that of dermis. Since we find the level of this enzyme to be quite low, however, it is possible that inter-species variation is also of importance.

The pH optima and the Michaelis–Menton constants of the epidermal glycosidases are compared with those of whole human skin and certain other mammalian tissues in Tables 2 and 3 respectively. The general similarities in properties between the epidermal enzymes and those described in isolated lysosomal preparations provide adequate confirmation that our enzymes are indeed of lysosomal origin. It is also of interest that epidermal β-galactosidase is relatively thermolabile (both during assay and storage), since a similar observation has recently been made regarding the corresponding lysosomal enzyme from liver (Meisler, 1972). The findings reported in this paper are thus compatible with the main body of evidence indicating that lysosomes play an important role in the dynamics of epidermal metabolism (Wolff & Schreiner, 1970).

Note added in proof. Our attention had been drawn to the report of Ohkawara *et al.* (*British Journal of Dermatology*, **87**, 450, 1972) which describes the measurement and characterization of a similar group of enzymes in human epidermis.

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British Journal of Dermatology (1975) **93**, 11.

Prealbumin bearing lymphocytes as an indicator of hapten protein carrier in certain varieties of dermatitis

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Accepted for publication 31 October 1974

SUMMARY

Patients with dermatoses where the antigen is a hapten, such as those with contact eczema and drug allergy, had high mean values of lymphocytes with membrane bound prealbumin detected by immunofluorescence methods.

These results support the hypothesis that lymphocytes bind prealbumin. We speculate that this was haptenized prealbumin.

Human prealbumin is a tryptophan-rich protein of comparatively low molecular weight. Its physiological role is not clear. It has been found to bind to certain biological substances such as thyroxin (Ingbar, 1958; Tata, 1961; Oppenheimer, 1965; Nayer *et al.*, 1966; Tritsch, 1972). Moreover it has been shown (Goodman & Leslie, 1972) that prealbumin binds with human plasma retinol-binding protein and this complex significantly stabilizes the retinol–retinol-binding protein complex. Because of the capacity of prealbumin to bind to certain biological substances and because a decreased level of prealbumin could be found in the blood of patients with Lyell's syndrome caused by drugs (Husz *et al.*, 1971) and in twenty-seven out of thirty-two cases of proved drug allergy (Husz *et al.*, 1974), it was assumed that prealbumin might act as a hapten protein carrier and thus may contribute to delayed or immediate hypersensitivity. The formation of an immunogenic conjugate between a simple chemical (or hapten) and a carrier molecule *in vivo* is an absolute requirement for the induction of an immune response towards a hapten.

In order to ascertain whether or not prealbumin plays some role in contact eczema and drug allergy, immunofluorescence studies were carried out to demonstrate the presence of prealbumin on the surface of lymphocytes.

MATERIALS AND METHODS

Patients with allergic contact dermatitis (ten) and drug reactions (ten) provided the lymphocytes.

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Normal healthy department staff members (ten) and patients with atopic dermatitis (ten) served as controls.

Lymphocyte separation

Lymphocyte separation was carried out by the method of Böyum (1968). 20 ml peripheral blood was collected by venepuncture in Erlenmeyer flasks containing fifteen glass perles, and shaken for 10 min. After defibrination, 60 ml isotonic solution was added. From this suspension 8 ml was added to 3 ml Ficoll–Isopaque solution and was centrifuged at 400 g for 20 minutes. The resultant lymphocyte-rich layer in between the Ficoll-Isopaque and the supernatant was aspirated, washed three times in isotonic NaCl solution, and lightly centrifuged at 200 g. The cells thus obtained were resuspended in isotonic NaCl solution so as to get about 5 million cells per ml, composed of about 90–95% lymphocytes and 5–10% monocytes and polymorphonuclear neutrophils.

Immunofluorescence staining and examination

The chemical and immunochemical data of the conjugates used are listed in Table 1.

TABLE 1. Characteristics of FTC-protein conjugates

Patient	Conjugates	Batch no.	FTC content (µg/ml)	Protein content (mg/ml)	F/P ratio (µg/mg)	Molar ratio (F/P × 0·411)	Antibody unitage	Dilution used for staining
1	Rabbit antihuman-pre-albumin/FTC	F 334 H*	91·0	18·[illegible]	4·9	2	4	1/32, 1/64
2	Rabbit antihuman-albumin/FTC	5–670†	17·6	7·[illegible]	2·3	0·94	2	1/32
3	Goat antirabbit/FTC GAR/FTC	3–171†	60·0	26·[illegible]	2·23	—	—	1/32, 1/64
4	Rabbit antiswine/FTC RASw/FTC	2–969†	18·3	15·[illegible]	1·17	—	—	1/32

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FTC content of conjugates was determined by the method of McKinney, Spillane & Pearce (1966) using fluorescein diacetate (FA) as reference standard. Protein content was measured by the method of Lowry *et al.* (1951).

Antibody 'unitage' of conjugates, using serum as a source of antigen, was determined as described by Beutner, Sepulveda & Barnett (1968).

Prealbumin staining could only be carried out on cell smears because the adding of FTC-labelled antihuman prealbumin antiserum to the cell suspensions caused their agglutination. By this method it is possible that intracellular as well as membrane 'bound' prealbumin is detected.

For the smear technique the slides were cleaned initially in 96% ethyl alcohol and subsequently treated in a gas flame. A smear was made from one drop of the lymphocyte suspension. After drying at room temperature for 15 minutes the smears were washed in phosphate buffer pH 7·2 and subsequently incubated for 30 min with the conjugate. The slides were washed twice in the buffer for 15 min before mounting with Difco mounting fluid.

In each case the percentages of prealbumin-positive lymphocytes were determined by examination of the microscope field alternately in blue narrow-band (480–490 nm) epi-illumination for fluorescence and in conventional light. Fluorescing cells in most instances showed a coarsely grained staining (Fig. 1). Trypan-blue viability of the cell suspensions utilized was greater than 97% and was performed before IF staining.

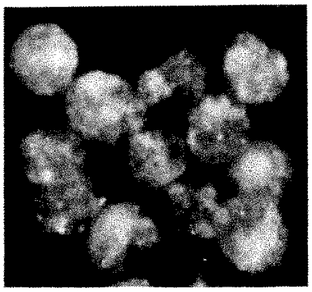

FIGURE 1. Specific prealbumin bearing lymphocytes in a patient with allergic contact dermatitis (Case 1). Direct immunofluorescence staining with rabbit anti-human prealbumin/FTC; dilution 1/32, magnification × 350.
Note: the bound prealbumin fluorescence is spotted rather than diffuse.

Specificity tests

Control tests were performed. Unconjugated prealbumin specifically inhibited the conjugated counterpart but did not inhibit staining due to conjugated antiserum directed against different classes of immunoglobulins. Incubation with heterologous antisera like rabbit anti-swine/FTC and goat anti-rabbit/FTC gave negative staining results. The addition of normal rabbit serum to the cell suspensions did not reduce the number of cells staining.

RESULTS

The distribution pattern of prealbumin bearing lymphocytes in healthy controls and in patients with various dermatoses is shown in Table 2.

The mean value of prealbumin bearing lymphocytes in the healthy controls was 4·5%; the range was 2·5–6%. Slightly higher mean values were noted in patients with atopic eczema (7·7%). Surprisingly, however, in two groups, each of ten patients, the mean values of lymphocytes with membrane-bound prealbumin were higher—in allergic contact dermatitis 28·3% and in drug reactions 22%.

DISCUSSION

A deficiency or a decrease of prealbumin level in the blood and its presence in the blister fluid of the patients with Lyell's syndrome have been demonstrated by Husz *et al.* (1971). These authors also reported a decrease of prealbumin level in twenty-seven out of thirty-two patients with proved drug allergy. These findings, together with the fact that prealbumin can form complexes with certain substances such as thyroxin and retinol-binding protein, lead to the assumption that this protein might be directly or indirectly involved in certain drug allergies. The possibility that this protein acts as a hapten protein carrier has been discussed in publications dealing with the examination of

TABLE 2. Distribution patterns in percentage of prealbumin bearing lymphocytes in various dermatoses

Patient no.	Healthy persons (%)	Atopic eczema (%)	Contact dermatitis (%)	Drug reactions (%)
1	5	6	49	8·5
2	2·5	8·5	28	8
3	6	5	23	15
4	6	3	60	18
5	5	4	17	25
6	4	25	15	20
7	5	4	22	54·5
8	4	9	6	20·5
9	5	6	8	22·5
10	3	6·5	55	27·5
Range	2·5–6	3–25	6–60	8–54·5
Mean value	4·5	7·7	28·3	22

antigen binding lymphocytes by auto-radiographic methods in animal experiments (Naor & Sulitzneau, 1967; Davie, Rosenthal & Paul, 1971; Rolley & Marchalonis, 1972; Donald, Morley & Beck, 1973).

The only patient (No. 6) with atopic eczema who had a high percentage of prealbumin bearing lymphocytes also had a contact allergy from a hair colouring product.

In those cases with contact dermatitis (Nos. 8 and 9) and drug reactions (Nos. 1 and 2) where the percentage of prealbumin bearing lymphocytes was not increased above the 10% level, the examination of lymphocytes was performed 1 or 2 weeks after the onset of the disease. Therefore it seems to be important to take blood from the patients at the very beginning of the disease or within 48 h after the exposure to antigens.

The fact that the bound protein fluorescence forms discrete spots or patches and is not diffusely smudged (Fig. 1) may indicate a 'switching on' of the lymphocyte by an antigen (Pernis *et al.*, 1970).

In almost all cases where the antigen is a hapten, such as patients with contact dermatitis or drug allergy, high mean values of lymphocytes with membrane bound prealbumin were noted in comparison to healthy controls and patients with atopic dermatitis.

These results indicate that prealbumin might be involved in the mechanisms of contact dermatitis and drug reactions but it is not known whether it acts as hapten carrier or not.

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British Journal of Dermatology (1975) **93**, 17.

The diagnostic significance of immunoglobulin and fibrin deposition in lichen planus

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Accepted for publication 31 October 1974

SUMMARY

Direct immunofluorescent (IF) staining was performed on biopsy specimens from fifty-three patients with active lichen planus. In fifteen of these cases uninvolved skin sites were also examined. Globular or cytoid body-like deposits of immunoglobulins, mainly IgM, were detected in forty-six of the active lesions, and in half the uninvolved skin biopsies. The deposition of fibrin in the papillary dermis and around follicular structures was seen only in the active lichen planus papules.

The significance of these findings was assessed by comparison with the IF results obtained in 252 biopsies from various cutaneous disorders, stained by the same technique during the period of this study. Although the presence of immunoglobulin cytoid bodies and fibrin was found to be highly characteristic of lichen planus, these findings were not specifically diagnostic. Morphologically identical deposits were seen not infrequently in lupus erythematosus and in eczema. Active lesions of dermatitis herpetiformis, erythema multiforme and other rare dermatoses also showed these cytoid body-like immunoglobulin deposits.

In addition to the immunoglobulin and complement band deposits at the basement membrane in lupus erythematosus (LE), several investigators have also described globular or cytoid body (CB) deposits of these materials in the dermal papillae (Burnham & Fine, 1969; Tan & Kunkel, 1966; Ueki, 1969; Baart de la Faille-Kuyper & Cormane, 1968; Pohle & Tuffanelli, 1968). Similar CB deposits have been seen in dermatomyositis (Winkelmann, Jordan & de Morogas, 1972) and their frequent or constant presence in lichen planus (LP) has also been described (Ueki, 1969; Michel *et al.*, 1970; Waisman, Dundon & Michel, 1973; Michel & Sy, 1973; Baart de la Faille-Kuyper & Baart de la Faille, 1974).

Much attention has also been directed to the deposition of fibrin products in LP (Barthelmes & Haustein, 1970; Salo, Tallburg & Mustakalio, 1972; Ebner & Kraft, 1972, 1973; Waisman *et al.*, 1973; Michel & Sy, 1973; Baart de la Faille-Kuyper & Baart de la Faille, 1974). We have attempted to investigate the diagnostic significance of these cytoid body and fibrin deposits in a number of inflammatory dermatoses.

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MATERIALS AND METHODS

Biopsy specimens of fifty-three cases of LP were divided, half rapidly frozen in liquid nitrogen and half fixed in 10% formol saline for routine histological analysis. Frozen tissue was stored at −70°C and cut at 5 μm in a cryostat at −25°C within 1 week of biopsy. In fifteen cases, uninvolved skin was obtained from sites distant from the areas of active LP and treated in the same way. During the period of this investigation 252 other skin biopsy specimens were obtained for diagnostic purposes and processed identically using the same conjugated antisera. Direct IF staining was performed with commercially obtained fluorescein labelled conjugated antisera as previously described (Abell, Black & Marks, 1974). These conjugates had the following properties:

Sheep anti-human IgG F/P ratio 4·1 molar Ab.P 0·71 mg/ml 4 units/ml, diluted 1:30 (Wellcome).
Sheep anti-human IgM F/P ratio 3·4 molar 2 units/ml, diluted 1:20 (Wellcome).
Sheep anti-human IgA F/P ratio 3·3 molar 1 unit/ml, diluted 1:10 (Wellcome).
Goat anti-human C_3 (B1a,B1c) F/P ratio 2·1 molar 8 units/ml, diluted 1:30 (Hylands).
Rabbit anti-human Fibrin F/P ratio 2·2 molar Prescription titre 1:16, diluted 1:30 (Hylands).
Rabbit anti-human Albumin F/P ratio 1·7 molar diluted 1:20 (Nordic).

The sections were mounted in buffered glycerol at pH 8.

The specificity of the antiglobulin conjugates had been determined by gel diffusion and by direct IF staining of monoclonal bone marrow preparations and had been shown to be heavy chain specific. Specificity of the remaining conjugates was demonstrated by immuno-electrophoresis. Negative and positive control specimens were incorporated in each experiment and in six cases staining with fluorescein isothiocyanate (FITC) dissolved in phosphate buffered saline at dilutions of 0·1% to 0·0001% was also performed. Blocking experiments were performed on nine active LP lesions using unlabelled sheep anti-human IgG and IgM (Wellcome) antisera (unit assay of both 8 units/ml). Blocking was attempted with these anti-sera undiluted and diluted to 1 part in 10, 20 and 40. A Leitz SM microscope illuminated by a HBO 200 mercury vapour lamp was used with primary filters Bg 38 (red) and KP490 and with secondary filters K510 or K530. The formalin fixed tissues were routinely stained with haematoxylin and eosin (H & E), periodic acid Schiff (PAS) and alcian blue, H & E pH 2·5.

RESULTS

Histological changes of LP were confirmed in all fifty-three cases. In none of the biopsies from uninvolved sites was evidence of LP activity detected.

Immunoglobulin CB deposits were detected in forty-six of the fifty-three active LP papules (Fig. 1). All three major immunoglobulin classes were detected on these bodies (Table 1) but IgM was detected in all positive cases and produced the most intense fluorescence. Complement C_3 could not be specifically identified in any of these CB deposits, although in a single case deposition in the walls of

TABLE 1. IF Ig cytoid bodies and fibrin in lichen planus

	IF Ig cytoid bodies					
	Total cases	IgM	IgG	IgA	C_3	Fibrin
Lichen planus papules	53	46	14	7	—	47
Uninvolved skin	15	8	—	2	—	—

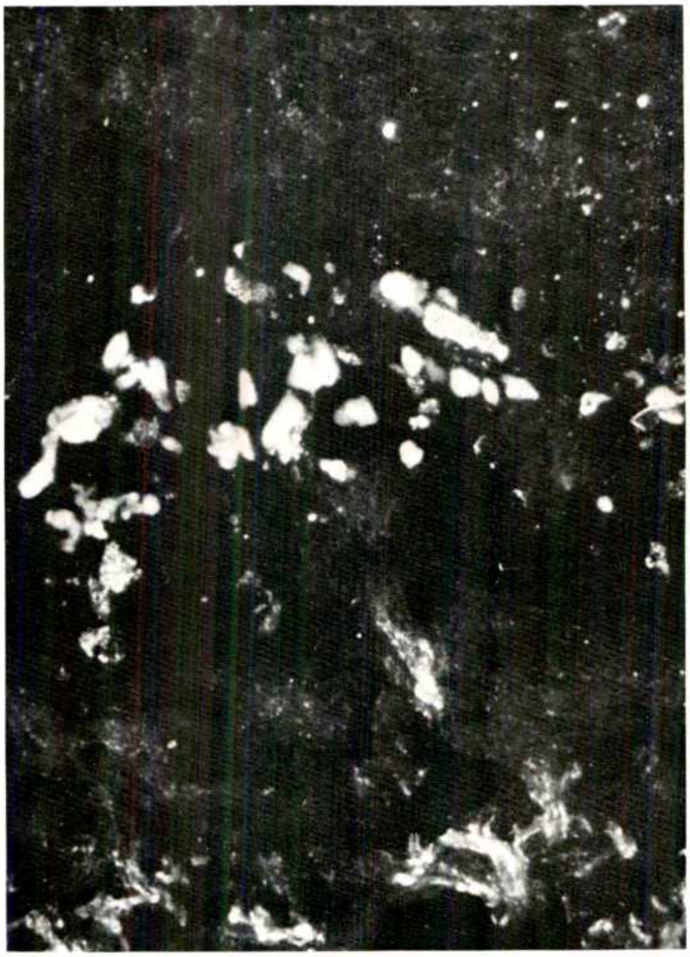

FIGURE 1. Lichen planus papule: direct IF IgM. Note cluster of cytoid body deposits in dermal papilla (× 300).

several papillary vessels was noted. Considerable variation in size was often apparent but few were much larger than a basal epidermal cell. Their numbers also varied markedly. Most lesions produced many CB deposits which tended to form clusters or aggregates. Less frequently only an occasional body would be found or serial sections might be required to demonstrate any.

The majority of these deposits lay in the papillary dermis adjacent to the epidermo–dermal junction of the surface or follicular epithelium. Intra-epidermal localization was occasionally seen and sometimes they were found in the reticular dermis apparently unrelated to follicular structures. In two specimens a finely stippled pattern of IgM was seen in the BM zone but only in the papillae containing CB deposits.

Examination of the fifteen biopsies from uninvolved skin revealed IgM CB deposits in eight. They were set singly or in small groups in the papillary dermis close to the epidermis. Deep or perifollicular bodies were not found.

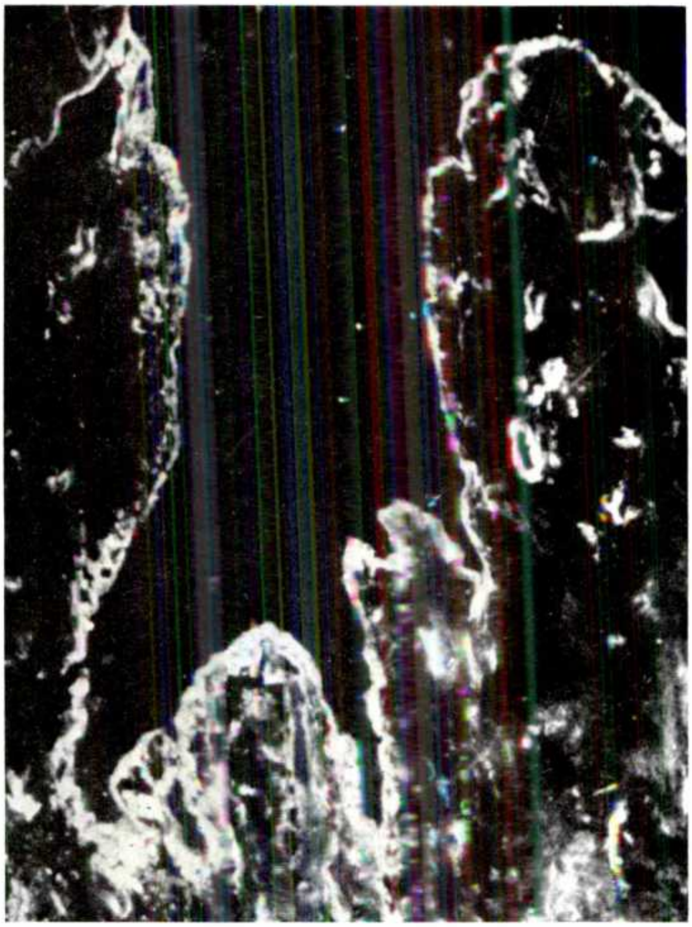

FIGURE 2. Lichen planus papule: direct IF fibrin. Note the junctional and papillary deposition (× 125).

Fibrin deposition occurred in all the biopsies in which cytoid bodies were found and in two in which they were absent. In none of the uninvolved biopsies could fibrin be detected. The pattern of fibrin deposition was not uniform. In all cases it was found deposited evenly against the epidermo-dermal junction occasionally producing a thin homogeneous band, but more often irregular strands extended from the junctional band into the papillary or perifollicular dermis (Fig. 2). The fibrin staining frequently demonstrated the CB deposits in both the epidermis and dermis and occasionally fibrin was detected in or around papillary vessels. In two biopsies weak staining of CB was seen with the anti-albumin conjugate but FITC staining was always negative.

Prior incubation with the undiluted unconjugated antihuman IgM serum produced complete blocking in only one of the nine biopsies tested. In the remaining eight, substantial but incomplete blocking was achieved but this was rapidly lost by diluting the blocking antisera.

Blocking of IgM CB staining could not be produced by the unlabelled IgG conjugate although this did succeed in complete blocking of the weaker IgG CB deposits.

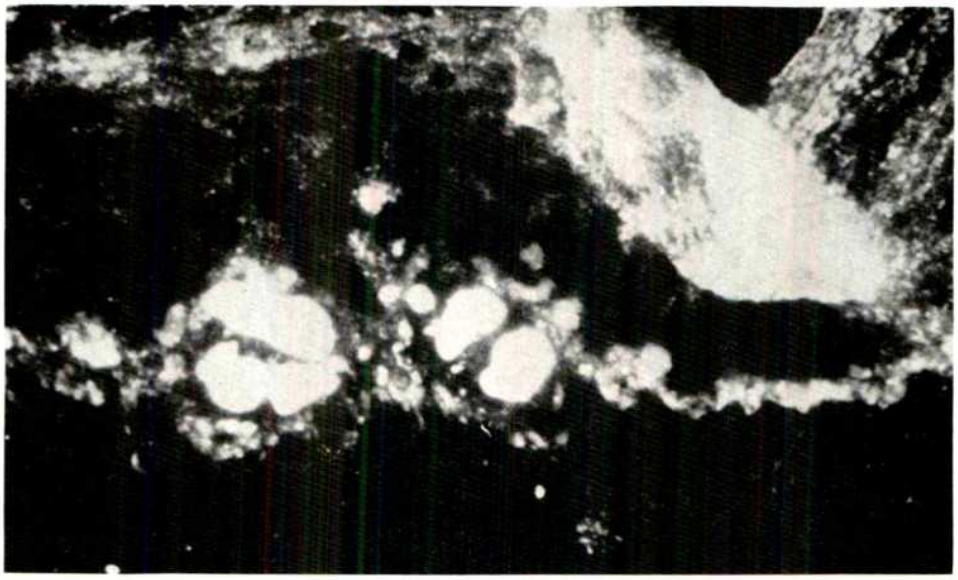

FIGURE 3. Lupus erythematosus: direct IF IgM. Note both the LE band of immunoglobulin and the large cytoid body deposits (× 500).

TABLE 2. IF Ig cytoid bodies in other dermatoses

	Total	CB Ig
Lupus erythematosus	49	11
Eczema	44	11
Dermatitis herpetiformis	9	4
Erythema multiforme	3	2
Lichen sclerosus et atrophicus	3	1
Drug erythema	2	1
Degos' malignant papulosis	1	1
Lymphomatoid papulosis	1	1
Chilblain	1	1
Uninvolved (normal) skin	—	4

CB deposits morphologically indistinguishable from those of LP were seen in eleven of forty-nine biopsies (Table 2) of cutaneous LE (Fig. 3). The IF staining reaction was identical, but in some sections the staining was more intense. In LE the tendency of these bodies to form large clusters was less pronounced, and they were never very profuse in number.

In eight of these eleven positive cases (LE) IF bands of immunoglobulin and complement were also present but in three clinically and histologically typical cases, this LE band was absent. Although complement C_3 staining of the 'LE' band was well demonstrated in these eight positive cases, C_3 could not be detected on the CB deposits. Fibrin deposition at the epidermal junction was also present in nine of the eleven cases, generally as a thick homogeneous band (Fig. 4).

Forty-four biopsies with eczematous histology produced eleven sections showing CB deposits.

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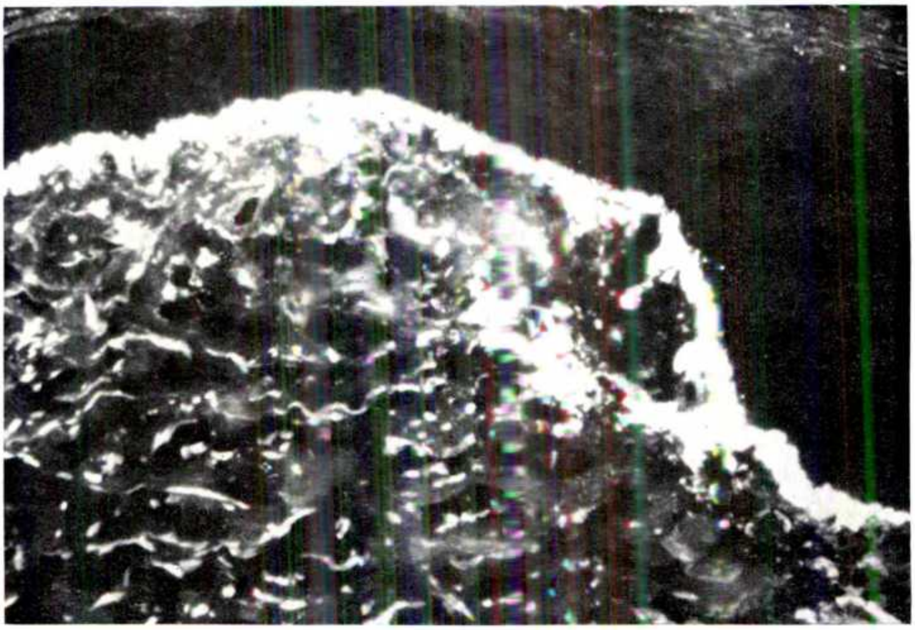

FIGURE 4. Lupus erythematosus: direct IF fibrin. Note the thick homogeneous fibrin deposit at the dermo–epidermal junction (× 125).

They were never numerous; always in the papillary dermis either singly or in small groups. Diffuse dermal fibrin deposition was not uncommon but never simulated the patterns seen in LP or LE.

Clusters of IF cytoid bodies were also seen in bullous lesions of dermatitis herpetiformis and erythema multiforme. Small numbers of solitary CB's were seen in single cases of a drug eruption (sulphinpyrazone), chilblains, and in the apparently uninvolved skin from single cases of generalized eczema, dermatitis herpitiformis, cutaneous LE, and nodular prurigo. Clusters of IgM cytoid bodies and papillary fibrin deposition similar to that seen in LP were found in a case of lichen sclerosus et atrophicus and also of malignant atrophic papulosis (Degos). A biopsy from a case of lymphomatoid papulosis (to be reported elsewhere) showed vast numbers of these CB deposits extending throughout the deep dermal infiltrate.

DISCUSSION

Our results differ in a number of ways from those of previous investigators. We have been unable to detect the presence of IgM or complement C_3 deposition at the basement membrane zone, as described by Baart de la Faille-Kuyper & Baart de la Faille (1974); we did observe in two biopsies localized deposition of IgM at this site, but only in the papillae in which the CB deposits were also found. Our results did, however, support their finding that the major immunoglobulin found on the CB's is IgM.

Although, occasionally the CB's could be visualized with the anti-C_3 complement conjugate, we could not detect specific C_3 fixation on these bodies in any of the dermatoses studied. Of particular note was the absence of C_3 staining of the cytoid bodies in sections of discoid LE in which C_3 could be readily detected in the BM band.

Reports of fibrin deposition in LP have given more uniform results. We were not able to detect such well developed fibrin deposition in the vessels of early lesions as described by Barthelmes & Haustein (1970). Fibrin does not appear in the uninvolved skin biopsies.

The incomplete blocking of IgM staining of the CB's by an unlabelled IgM antiserum of twice the unitage of the conjugated IgM antiserum was seen not only in LP but also in LE, eczema, lymphomatoid papulosis and in the uninvolved LP biopsies. The IgM in the BM band of LE lesions was completely blocked, while only partial blocking of the IgM of the CB's could be obtained. Despite this, the reduction of IF staining was substantial and was considered to indicate a high degree of specificity. Blocking of IgG staining in all circumstances was complete, and staining of CB's with free fluorescein could not be demonstrated.

The presence of CB deposits was not diagnostic of LP, but large clusters were certainly highly characteristic of this condition, as also was the pattern of fibrin deposition in the papillae. LE and eczema showed similar but fewer CB's.

In most cases clinical and histological evaluation will prevent diagnostic errors between LE and LP although an occasional case may give rise to difficulty, especially with facial lesions. We have had such problems in three cases of facial LE which failed to show a BM IF band of immunoglobulin, but in which IF CB's suggested the possible diagnosis of LP. The histology in all three cases was characteristic of LE however. We did not find deposits of IgM or complement at the BM zone in cases of LP which have morphologically suggested LE.

Michel and Sy's series included two cases of probable drug induced LP. One case in this present report was confirmed to be due to practolol by a successful provocation test, and the IF findings were identical to those seen in idiopathic cases.

Thyresson & Moberger (1957) postulated that the primary disorder in LP involves degeneration of basal epidermal cells, and the production of hyaline or colloid bodies. Black (1972) considered that the histological changes in LP are in part due to the migration of epidermal cells into the areas of basal cell damage, and histochemical (Black & Wilson Jones, 1972) and radiographic (Marks, Black & Wilson Jones, 1973) evidence has been obtained to support these concepts. Ueki considered that the IF globular or CB bodies are located on the hyaline bodies, and subsequent reports have supported this view.

Hyaline bodies are best stained by PAS after diastase digestion (Goltz & Hult, 1963) but it was rarely possible in the formalin fixed tissue to find hyaline bodies in equivalent numbers to the IF CB's seen in frozen sections of the same tissue. Unfortunately, PAS staining of frozen sections, and restaining of sections used for IF did not clearly define hyaline bodies. Hyaline bodies were seen in some PAS sections of LE but were not found in eczema or uninvolved skin.

Peroxidase labelled antiglobulin preparations examined by electron microscopy might further elucidate this apparent discrepancy. At the present time it still remains to be proved that hyaline body formation is responsible for all the cytoid bodies seen in IF preparations in these dermatoses.

The finding of these IF bodies in so many dermatoses, as well as in apparently uninvolved skin, suggests that this change might well represent a non specific phenomenon of basal epidermal cell degeneration, caused by a number of differing processes.

The interpretation of the part played by these immunoglobulin and fibrin deposits in LP and other conditions also remains to be determined.

ACKNOWLEDGMENTS

We wish to acknowledge the help of Mr S.J.Robertson and Mrs M.E.Manley in producing the photomicrographs.

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British Journal of Dermatology (1975) **93**, 25.

Deficiency of circulating IgA and IgM in adult patients with lichen planus

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Accepted for publication 4 November 1974

SUMMARY

Investigations on thirteen consecutive adult patients with classical lichen planus revealed a significant lowering in the levels of circulating IgA and IgM. Patients with lichen planus may have an immunological defect which makes them susceptible to infection with an unknown virus.

Despite much recent investigation the cause of lichen planus remains unknown (Black, 1972). The development of a lichen planus papule has been attributed to a viral infection (Swanbeck & Thyresson, 1964; Depaoli, 1953), to an inborn error of metabolism (Cotton, van den Hurk & van der Staak, 1972) or to continuous minute damage of unknown origin to the cells in the basal layer of the epidermis (Marks, Black & Wilson Jones, 1973; Presbury & Marks, 1974). Black (1972) stated that there is very little evidence for an autoimmune cause for lichen planus, but Sarkany & Gaylarde (1972), in a preliminary investigation, reported *in vitro* lymphocyte transformation in two of six patients with lichen planus when their lymphocytes were cultured in the presence of a homogenate made from a lesion taken from the patient's own skin; and Lachapelle & De la Brassine (1973) used autoradiographic and cytochemical techniques on lichen planus lesions and found the cells in the dermal infiltrate to be proliferating lymphoblasts. The latter workers suggest that this blast transformation could represent an immunological response.

There has been no report on levels of circulating immunoglobulins in patients with lichen planus. This is such a report on thirteen patients.

METHOD

Thirteen unselected consecutive patients with classical lichen planus in various stages of activity took part in the investigation. Blood was taken from each patient and the immunoglobulin levels were estimated by single radial immunodiffusion according to Mancini, Carbonara & Heremans (1965).

RESULTS

The results of this investigation are shown in Table 1. A statistical analysis of these results (Table 2)

TABLE 1. Immunoglobulin levels in thirteen patients with lichen planus

Patient	Sex	Age	Sites	Lesions in mouth	Activity	IgG (700–1800 mg%)	IgA (90–450 mg%)	IgM (90–280 mg%)
1	F	72	Generalized	–	Active	760	88	152
2	F	44	Generalized	–	Settled	1200	152	56
3	M	30	Limbs	+	Active	1520	60	60
4	M	68	Generalized	–	Settled	1180	200	60
5	F	54	Arms & chest	–	Settled	1760	160	60
6	M	41	Generalized	–	Settled	1040	116	96
7	M	72	Limbs	–	Settled	1600	120	80
8	F	51	Limbs	–	Settled	1240	112	84
9	F	43	Legs	–	Active	1060	48	228
10	F	58	Generalized	–	Settled	720	40	136
11	M	48	Penis	+	Active	1120	60	56
12	F	66	Generalized	+	Settled	995	68	306
13	F	56	Generalized	+	Active	1160	82	40

TABLE 2. Statistical analysis of immunoglobulin levels

Factor	Normal*		13 patients with L.P.		Difference of means	
	Mean	s.d.	Mean	s.e.	t	Significance
IgA	270	45	98·2	48·1	12·4	$P<0{\cdot}001$
IgM	170	27·5	102·2	61·9	3·8	$P<0{\cdot}01$
IgG	1250	137·5	1159·6	300·5		Not significant

* These values were derived from 150 hospitalized individuals, mostly admitted for elective minor surgery and having no disorder known to affect the immunoglobulin levels (Dr F. Albert-Recht, Department of Chemical Pathology, University of Aberdeen).

shows that as a group the thirteen patients with lichen planus had a significant lowering of IgA and IgM. When considered individually for IgA all the patients had levels below the normal mean and seven had levels below the 95% probability limit; for IgM all the patients except two had levels below the normal mean and three had levels below the 95% probability limit. The IgA levels were depressed in all five patients with active lesions compared with two of eight patients in whom the disease had settled ($P<0{\cdot}05$). This depression of IgA cannot be attributed to generalized skin involvement which was present in only two of five patients with active disease (40%) compared with five of eight patients (63%) whose disease had settled.

DISCUSSION

This investigation shows a significant reduction in the circulating levels of IgA and IgM in patients with lichen planus. Immunoglobulin deposition is reported in both affected and unaffected skin of patients with lichen planus. IgM is found in the upper dermis (Baart de la Faille-Kuyper & Baart de la Faille, 1974) and in the basement membrane zone (Copeman, Schroeter & Kierland, 1970) of lichen planus lesions and IgG has been found in the clinically unaffected skin (Baart de la Faille-Kuyper, 1972). The demonstration of IgG, IgA and IgM in the colloid body—one of the earliest pathological changes—led Baart de la Faille-Kuyper & Baart de la Faille (1974) to the conclusion that immunoglobulins may play a primary role in the genesis of the colloid body.

The reduced blood levels of IgA and IgM in patients with lichen planus requires elucidation. Reduced IgA and IgM levels are present in the blood of patients with acquired dysgammaglobulinaemia type 1 (Gilbert & Hong, 1964) who have a history of susceptibility to infection between the ages of 19 and 29 years, and present with diarrhoea, malabsorption and infestation with Giardia lamblia (Adenolfi, 1969). There was no history of susceptibility to infection or bowel upset in this group of patients with lichen planus but the immunoglobulin levels were only slightly reduced. It is tempting to suggest that patients with lichen planus have an immunological deficiency and are susceptible to an unknown viral agent which may be released when the unaffected skin is subjected to damage (Stankler & Ewen, 1974).

ACKNOWLEDGMENT

I would like to thank Dr T.M.S.Reid, Immunology Unit, Department of Bacteriology, Medical School, Aberdeen, for helpful suggestions.

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The effects of catecholamine and related compounds on the adenyl cyclase system in the epidermis*

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Accepted for publication 4 November 1974

SUMMARY

The effect of various hormones and drugs on the adenyl cyclase system of pig and human epidermal slices was studied *in vitro.*

Adrenaline and isoproterenol in the presence of theophylline increased the epidermal cyclic AMP level 20-fold in 5 min. Noradrenaline also stimulated cyclic AMP accumulation but to a much lesser degree. The adrenaline stimulation was marked even in the absence of the phosphodiesterase inhibitor, theophylline. Theophylline potentiated the effect of adrenaline at the concentration of 2–10 mM although theophylline alone did not elevate the cyclic AMP level significantly. The Km for adrenaline stimulation of the adenyl cyclase system of pig epidermis was $7{\cdot}7 \times 10^{-7}$ M. A β-adrenergic antagonist, propranolol, markedly inhibited the adrenaline stimulation while α-antagonists, phentolamine or priscoline, showed little effect. These results are in accord with the view that the epidermis possesses an active adenyl cyclase system with β-adrenergic receptors.

Cyclic AMP metabolism has important consequences in every mammalian tissue (Robison, Butcher & Sutherland, 1971). Reported studies on skin on the effect of β-adrenergic drugs on cyclic AMP formation are shown in Table 1. Adenyl cyclase is a membrane enzyme which easily loses its hormone responsiveness during homogenization. As shown in Table 1 purified membrane fractions responded only poorly to stimulation, while epidermal slices incubated in a physiological medium and then subjected to catecholamine stimulation have responded much more physiologically. In this report, we describe the effect of adrenergic agents and related compounds on pig and human epidermal adenyl cyclase. Kinetic parameters thus obtained have helped to establish the 'optimal' experimental

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* Supported in part by Grant Am 17179 of National Institutes of Health.

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condition in regards to 'hormonal effectiveness', and should aid future experiments on the adenyl cyclase system of normal and diseased epidermis.

MATERIALS AND METHODS

Domestic pigs weighing 5–10 kg were used as experimental animals. The dorsal skin was shaved, wiped gently with water, and then removed as a sheet by use of a Castroviejo Keratome set at 0·4 mm. No anaesthesia was used, to avoid its possible effects on the experimental system. The human epidermal slices were obtained in the same way except that the thickness was 0·2 mm.

The removed epidermis was kept in Hank's balanced salt solution at 4°C and used within 1 hour.

The epidermis was cut into small pieces of approximately 6 × 6 mm, floated epidermal side up on Hank's salt solution and pre-incubated for 15–20 min at 37°C in room air. At the end of the pre-incubation period, two pieces of epidermis were transferred to 3 ml of incubation media consisting of Hank's salt solution with added experimental drugs and/or hormones and incubation was continued for a further 5 min at 37°C. At the end of the 5 min experimental period, the epidermal slices were removed from the medium and quickly frozen between flat dry ice. The frozen pieces were placed in test-tubes chilled in dry ice. After the addition of 500 µl of ice cold 0·1 N HCl, the tissue was boiled for 5 min and then homogenized. 300 µl of the homogenate was analysed for cyclic AMP content by the protein binding method of Gilman (1970) with a modification in extraction procedure as reported before (Yoshikawa *et al.* 1975).

Part of the remaining homogenate was used to measure protein content according to the method of Lowry *et al.* (1951).

RESULTS

(1) *Pre-incubation*

As reported previously, the cyclic AMP level rapidly increases during the first 5 min after removal of the skin and then slowly decreases (Yoshikawa *et al.*, 1975). ATP on the other hand decreases after removal (Kondo & Adachi, 1971).

It is, therefore, very important to bring the elevated cyclic AMP level down to physiological level prior to incubation with the drugs and hormones to be tested. This is the purpose of pre-incubating the skin.

As shown in Fig. 1, the elevated cyclic AMP level decreases quickly during pre-incubation at 37°C and within 15–20 min reaches its lowest level which is close to the physiological level. At the same time, the level of ATP, the substrate of adenyl cyclase, increases to reach the maximal and nearly physiological level at about 20–30 min.

After this period, the cyclic AMP level goes up again and the ATP level starts to decrease.

Based on these findings, the following experiments were carried out with pre-incubations of 15–20 min.

(2) *Incubation time*

After the pre-incubation, tissues were incubated in Hank's solution with 2 mM theophylline, 5×10^{-5} M adrenaline, and both together (Fig. 2).

With 2 mM theophylline alone, there was no significant increase of cyclic AMP during 15 min of incubation. 5×10^{-5} M adrenaline gave a rapid and immediate increase of cyclic AMP in the skin for the initial 5 min of incubation, thereafter the total cyclic AMP started to decrease despite the continuous presence of the adrenaline.

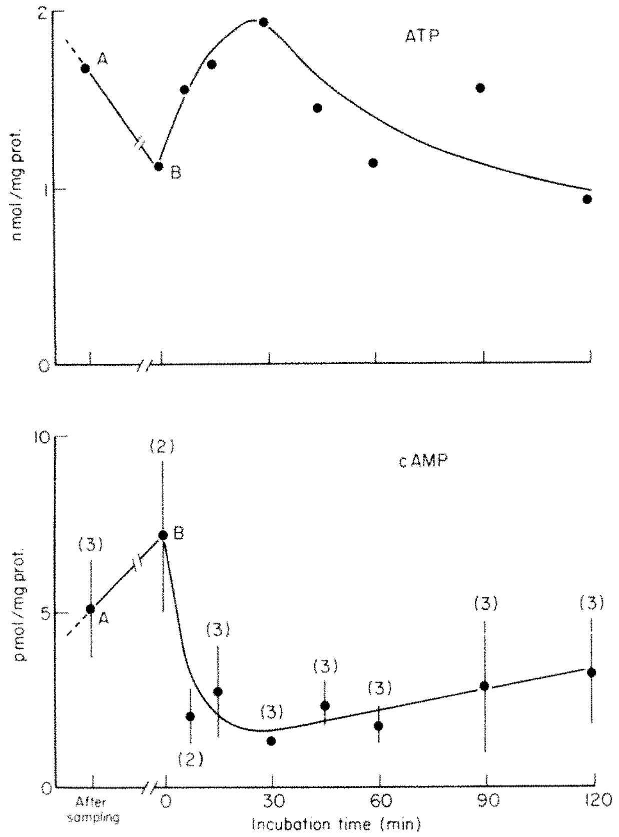

FIGURE 1. Effect of pre-incubation on the cyclic AMP and ATP content of the skin. Pieces of skin were incubated for the indicated periods in Hank's balanced salt solution at 37°C, frozen quickly and analysed for cyclic AMP and ATP content. ATP is the average of two separate experiments using one piece of skin for each time period in each experiment. It is measured by luciferine–luciferase bioluminescence method (Holmsen, Holmsen & Bernhardsen, 1966). Cyclic AMP is the average ± s.e. for two to three different series of experiments as indicated with two pieces of skin for each time period for each experiment. A is the concentration determined at the point where the skin specimens were left in air for about 30 s after the removal and then placed in Hank's solution for storage at 4°C. B is the concentration after 30–60 min of the storage at 4°C in Hank's solution, i.e. just before the beginning of the incubation at 37°C in fresh Hank's solution. Physiological concentration of ATP in the tissue is considered to be about 2 nmol/mg prot. (Kondo & Adachi, 1971), and that of cyclic AMP is 1–2 pmol/mg prot. (Yoshikawa *et al.*, 1975).

The addition of 2 mM theophylline potentiated the adrenaline effect, but the cyclic AMP level still reached a maximum after 5 min of incubation and decreased slowly thereafter.

The following experiments were therefore carried out with 5 min of incubation to see the maximum effects of the various agents.

(3) *Theophylline concentration*

The epidermal slices were incubated with various concentrations of theophylline in the presence and absence of adrenaline. The effect of theophylline alone was not significant even at 10 mM, while it remarkably enhanced the stimulatory effect of adrenaline on cyclic AMP accumulation (100%

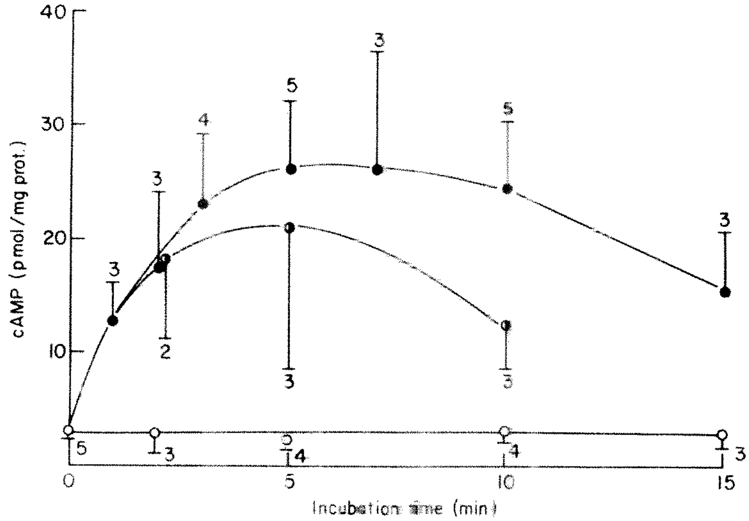

FIGURE 2. Change of cyclic AMP level during incubation period. Tissues were incubated for the indicated period with 2mM theophylline (○), with 5×10^{-5}M adrenaline (◐), and with both of them (●). The results are expressed as mean ± s.e.

increase in the cyclic AMP level at 10 mM theophylline after 5 min of incubation). Further experiments were done with 10 mM theophylline unless otherwise stated.

(4) *Effect of adrenaline concentration*

The epidermis was incubated with various concentrations of adrenaline ranging from 10^{-8} M to 10^{-4} M together with 10 mM theophylline for 5 min at 37°C (Fig. 3).

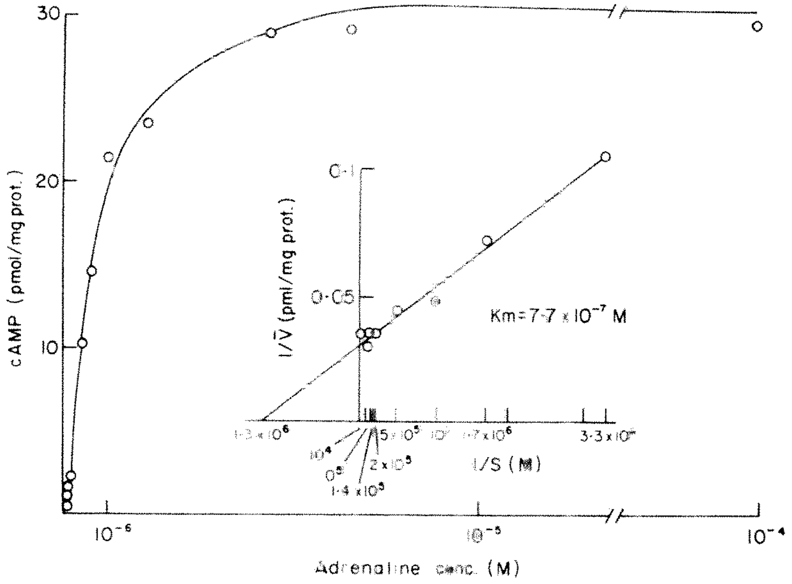

FIGURE 3. The effect of concentration. Tissues were incubated with various concentrations of adrenaline plus 10 mM theophylline.

Remarkable activation of adenyl cyclase by adrenaline was observed. The increase in cyclic AMP was linear up to an adrenaline concentration of about 5×10^{-7} M, then the rate of stimulation decreased. Maximal activation was obtained at a concentration of about 5×10^{-6} M. The apparent Km for adrenaline was $7{\cdot}7 \times 10^{-7}$ M and was in good agreement with those calculated for other tissues.

(5) *Effects of adrenergic agonist, antagonist and NaF*

The results are summarized in Fig. 4. Incubations were for 5 min at 37°C. As shown before, theophylline by itself had little effect, but did enhance the accumulation of cyclic AMP induced by adrenaline.

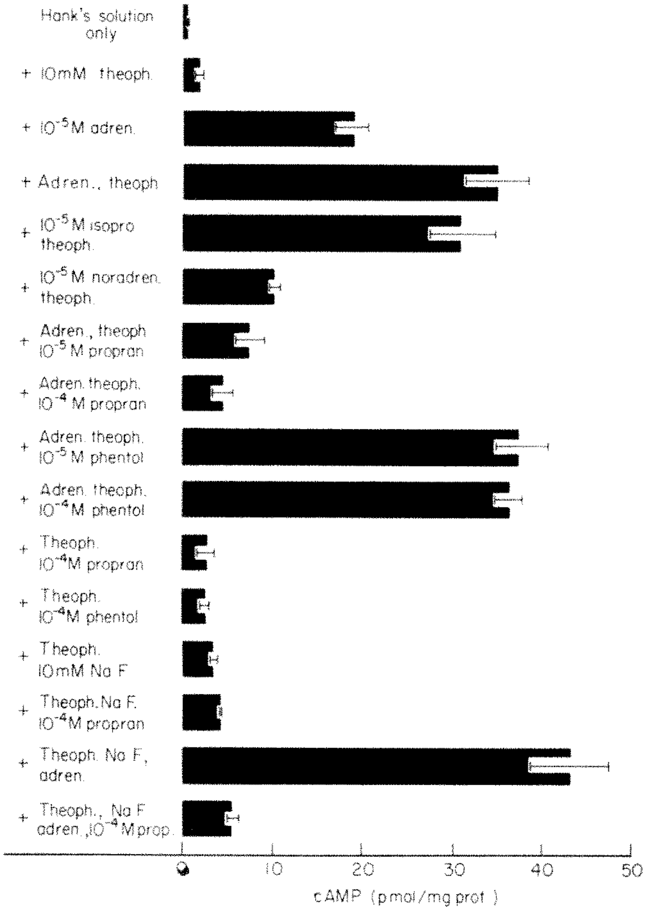

FIGURE 4. The effects of adrenergic agents, α and β-adrenergic blocking agents, theophylline and NaF on the cyclic AMP accumulation in pig skin. Data are expressed as the average and individual results in two different series of experiments as indicated by small bars. Two pieces of skin were used for each measurement.

Isoproterenol was found to be as effective as adrenaline in inducing cyclic AMP accumulation, while noradrenaline was not as effective.

Propranolol, the β-adrenergic blocking agent, markedly counteracted the stimulatory effect of adrenaline although its blocking effect was not complete at a concentration of 10^{-4} M, ten times higher than that of adrenaline. On the other hand, phentolamine, the α-adrenergic blocking agent, was ineffective in blocking the effect of adrenaline. Sodium fluoride (10 mM) increased cyclic AMP additively to the effect of adrenaline. The NaF effect, however, was small.

(6) *Studies on human epidermal slices*

The effects of theophylline, adrenaline, propranolol and priscoline (an α-blocker) were studied with human epidermal slices (Fig. 5).

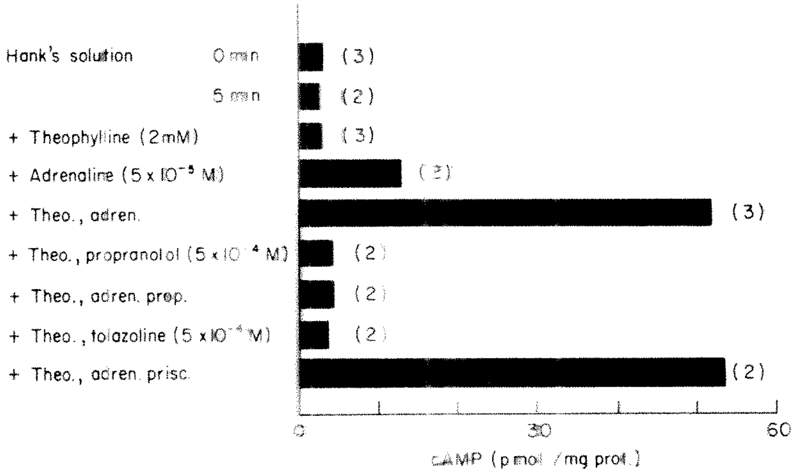

FIGURE 5. Effects of adrenaline, theophylline and α and β-adrenergic blocking agents on cyclic AMP accumulation in human skin. Data are the average of the results obtained with two to three different white adult skins, two pieces of which were analysed for each experiment.

The results were essentially the same as those with pig skin.

DISCUSSION

Previous data on catecholamine stimulation of epidermal adenyl cyclase are summarized in Table 1 Our data show the most remarkable stimulation by both adrenaline and isoproterenol. One reason for this clear-cut demonstration may in part be due to our pre-incubation of epidermal slices to decrease (and also to standardize) the cyclic AMP level, which invariably has risen after removal of the skin (cf. Fig. 1).

Marks & Grimm (1972) also reported marked stimulation (minimum 1000%) of cyclic AMP accumulation in skin with isoproterenol given intraperitoneally. Their studies were completely *in vivo* so that intra-epidermal cyclic AMP levels were low (and standardized) at the beginning of each experiment. Mui, Hsia & Halprin (1975) have also reported marked stimulation using a similar pre-incubation period.

Pig and human epidermis may also respond well because they consist of several layers of epidermal cells, unlike rodents' which consists of less than two layers. Consequently with rodents' epidermis it is difficult to avoid moderate to heavy contamination by dermis which contains both inert (collagen) and possibly antagonistic (blood vessels which contain α-receptors) elements. Another reason for the varying degree of stimulation reported is the use of a phosphodiesterase inhibitor. When the inhibitor, theophylline, was not added, only 25% of the stimulation by adrenaline in the presence of

TABLE I. β-agonist stimulation of epidermal adenyl cyclase

References	Samples	β-Agonist	Stimulation (%)	Remarks
Mier & Urselmann 1970	Guinea-pig whole skin	Adrenaline	0	*In vitro* homogenate
Brønstad *et al.* 1971	Hamster whole skin	Adrenaline	1500	*In vitro* slice ^{14}C-ATP pre-labelled
Powell *et al.* 1971	Rat epidermis	Isoproterenol	300	*In vitro* slice
Duell *et al.* 1971	New born rat whole skin	Isoproterenol	40	Particulate (17,000 × g)
Marks & Rebien 1972	Mouse whole skin	Adrenaline Isoproterenol	20–50	Particulate (1200 × g)
Marks & Grimm 1972	Mouse whole skin	Isoproterenol adrenaline noradrenaline	1000 300 150	*In vivo* experiment
Hsia *et al.* 1972	Human epidermis	Adrenaline	40	Particulate (650 × g)
Wright *et al.* 1973	Human epidermis	Adrenaline	300	*In vitro* slice ^{3}H-ATP pre-labelled
Voorhees *et al.* 1973	Human epidermis	Isoproterenol	100	*In vitro* slice no theophylline
Mui *et al.* 1975	Human epidermis	Adrenaline	1600	*In vitro* slice ^{3}H-ATP pre-labelled
Yoshikawa *et al.* (this paper)	Human & pig epidermis	Adrenaline & isoproterenol noradrenaline	2000 500	*In vitro* slice

theophylline was observed. No phosphodiesterase inhibitor was added in Voorhees *et al.* (1973) experiments, which showed only 100% stimulation.

Despite the varying degree of β-agonists' stimulation on adenyl cyclase of skin, all previous data and ours are in accord with the view that mammalian epidermis possesses an adenyl cyclase system with a β-receptor. Under optimal experimental conditions either adrenaline or isoproterenol can induce an accumulation of cyclic AMP in 5 min which is 20-fold higher than the initial value.

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The effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria

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Accepted for publication 1 November 1974

SUMMARY

Seven patients undergoing treatment for cutaneous hepatic porphyria had serial measurements of their plasma uroporphyrin. In each case there was a progressive fall during treatment but even in remission the levels did not return to normal. There was a significant correlation between urinary and plasma uroporphyrin concentrations.

There is, at present, no convenient and reliable method of assessing disease activity in cutaneous hepatic porphyria (porphyria cutanea tarda, CHP). Clinical manifestations are influenced by many factors, especially exposure to sunlight and trauma. To diagnose CHP detection of high urinary uroporphyrin excretion is essential; but quantitative measurements rely on accurate 24 h collections, which present difficulties in out-patients.

Patients with untreated CHP have increased quantities of uroporphyrin in their plasma (Moore *et al.*, 1973). This is secondary to increased uroporphyrin production by the liver which causes the high urinary uroporphyrin excretion and contributes to the cutaneous signs typical of the disease.

The purpose of this study was to determine whether a direct relationship exists between plasma uroporphyrin levels and disease activity.

PATIENTS AND METHODS

Fifteen patients with untreated, active CHP were studied. Their clinical details are summarized in Table 1. Twenty control plasmas were obtained from healthy, age and sex matched blood donors

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A paper based on this work was read to the Investigative Group of the British Association of Dermatologists at Liverpool, January 1974.

TABLE I

Patient	Age	Sex	Symptoms						Duration at first visit (months)	Serum iron (mg/100 ml)	Urinary uroporphyrin before treatment (μg/24 h)	Plasma uroporphyrin before treatment (μg/100 ml)	Suspected cause of porphyria	Comment
			Blisters	Erosion	Scars	Fragility	Pigmentation	Hypertrichosis						
Normal values										80–160	0–41	0–0·13		
J.C.	64	M	+	+		+			24	132	3,204	6·0	Alcohol	*
D.M.	68	M	+	+	+	+			2	114	4,407	31·7	Alcohol	*Also had ileostomy (ulcerative colitis)
S.C.	75	F	+	+	+		+	+	2	123	8,402	44·5	Unknown	Cholecystectomy for gall stones 12 months previously
J.B.	52	M	+	+	+	+			12	172	384·3	3·7	Alcohol	*
E.N.	38	F	+	+		+		+	2	133	11,582	11·2	Alcohol plus contraceptive pill	*
W.R.	73	M	+	+					3	118	1,923	3·9	Unknown	
W.W.	41	M	+	+	+	+			40	236	5,933	8·9	Alcohol	*
A.W.	47	M	+	+	+	+			1	195	3,081	4·2	Alcohol	
J.H.	64	M		+	+						420·1	2·2	Alcohol	*
W.D.	68	M	+	+	+	+			84	190	1,020	3·4	Alcohol	*
G.P.	58	M	+	+	+	+	+		24	141	5,536	10·8	Alcohol	Died of carcinoma of bronchus
J.T.	61	M		+		+	+	+	3	164	2,225	5·7	Alcohol	
A.H.	65	M	+	+	+	+			2	148	2,462	11·8	Alcohol	
R.A.	62	M	+	+		+	+		48	150	1,066	4·04	Alcohol	
J.McR.	71	M	+	+	+	+	+	+	2	170	1,326	3·1	Hepatoma	Died 2 months later

* Patients in whom serial measurements were made.

whose blood was being used for packed cells. Urinary uroporphyrin was measured by the method of Rimington (1961) and plasma uroporphyrin by the method of Moore *et al.* (1973). Full red cell, plasma, urinary and faecal porphyrin analyses were carried out in each patient but this paper deals only with uroporphyrin which showed the most marked and consistent abnormality.

Seven of the patients had regular measurements of 24 h urinary uroporphyrin and plasma uroporphyrin throughout the course of their therapy. Two were treated by venesection and five by plasmapheresis. All were strongly advised to give up alcohol. It was impossible to assess accurately whether this advice was being acted upon, but the problem was approached in two ways; direct questioning about consumption and blood alcohol measurements. All patients attended for treatment between 14.00 and 15.00 hours and some of the blood removed was examined for ethanol by gas chromatography using Porapak Q polymer beads.

Remission was judged to have occurred when there was absence of blistering with a marked reduction in skin fragility, as assessed subjectively, and a fall in urinary uroporphyrin to near normal levels. In patients undergoing venesection there was also a drop in serum iron, mild anaemia or a rise in erythrocyte protoporphyrin.

RESULTS

Most patients claimed to have reduced their alcohol consumption. W.D. admitted to drinking beer in the evenings but alcohol was never detected in his blood. D.M. and J.H. repeatedly showed significant quantities of alcohol (>5 mg/100 ml) in their blood (Table 2) but claimed they had stopped drinking.

Fig. 1 shows the plasma uroporphyrin levels in the fifteen untreated patients compared with the twenty controls. There is a marked elevation in the patient group ($P<0{\cdot}05$). The mean figure for patients is 10·35 μg/100 ml and the mean for controls is 0·03 μg/100 ml.

Fig. 2 shows a comparison of the plasma uroporphyrin concentrations before and after treatment

TABLE 2. Serial plasma uroporphyrin measurements (μg/100 ml) in seven patients undergoing treatment. All patients were advised to abstain from alcohol. Alcohol was detected in the blood samples marked with an asterisk

Patient	J.C.	D.M.	J.B.	E.N.	W.W.	J.H.	W.D.
Method of treatment	P	P	P	V	V	P	P
	6·0	31·7	3·7*	11·2	8·0	2·2	3·4
	4·6	18·6	1·7	7·6	8·9	2·1*	2·3
	6·3	14·8	0·2	9·3	7·6	2·2	3·0
	5·3	3·6	0·2	6·9	1·4	1·3	0
	2·4	2·8		5·4	5·4	1·4*	2·4
	1·7	2·6*		2·3	4·7	2·0	1·1
	1·6	1·8		0·7	5·1	0·4	1·4
	1·6	1·2*			1·5	1·7*	1·0
	1·1	0·4*			3·2	1·4*	0·8
	0·8				1·5		0·3
					1·9		1·0
					0·3		
Total no. of treatments	22	20	9	9	23	18	12

V, Venesection; P, Plasmapheresis.

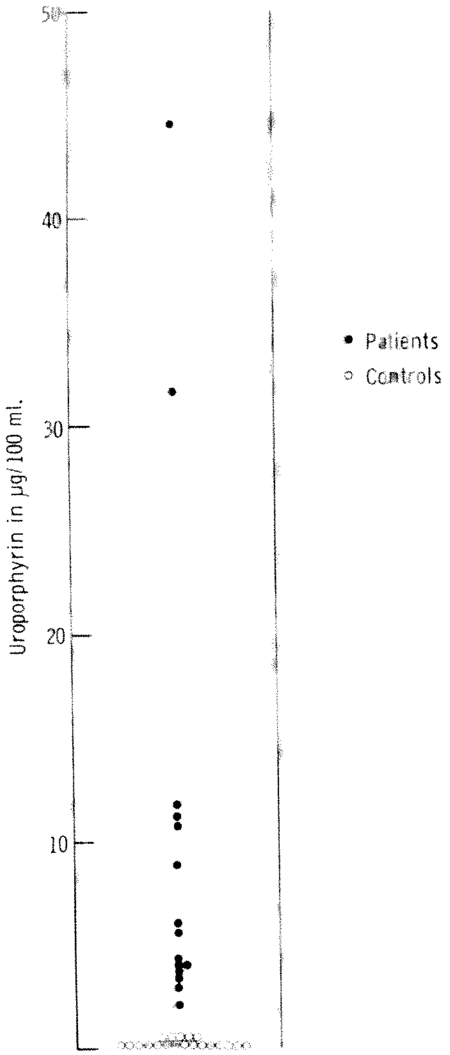

FIGURE 1. Plasma uroporphyrin levels in fifteen untreated patients compared with twenty controls.

in the seven patients in whom serial measurements were made. There is a significant drop during treatment ($P<0{\cdot}02$). Table 2 shows all the figures obtained during the course of therapy. There is some fluctuation in the values, but in all cases a downward trend is readily discernible.

No patient with visible manifestations of the disease had a uroporphyrin level of less than 2·2 μg/100 ml. No patient in full remission at the end of treatment had a level greater than 1·0 μg/100 ml, but even at that time the patients had a higher plasma uroporphyrin level (range 0·2–1·0, mean 0·54) than the control group (range 0–0·13, mean 0·03).

It is probable that the lack of consistent improvement in J.H. was due to continued alcohol consumption.

Analysis of the figures given in Table 1 for urinary and plasma uroporphyrin shows a highly significant correlation to a regression curve of form: Plasma uroporphyrin $= 2{\cdot}596\ e^{0{\cdot}0003\ \text{Urinary Uroporphyrin}}$ (regression coefficient (r) 0·85 $P<0{\cdot}001$).

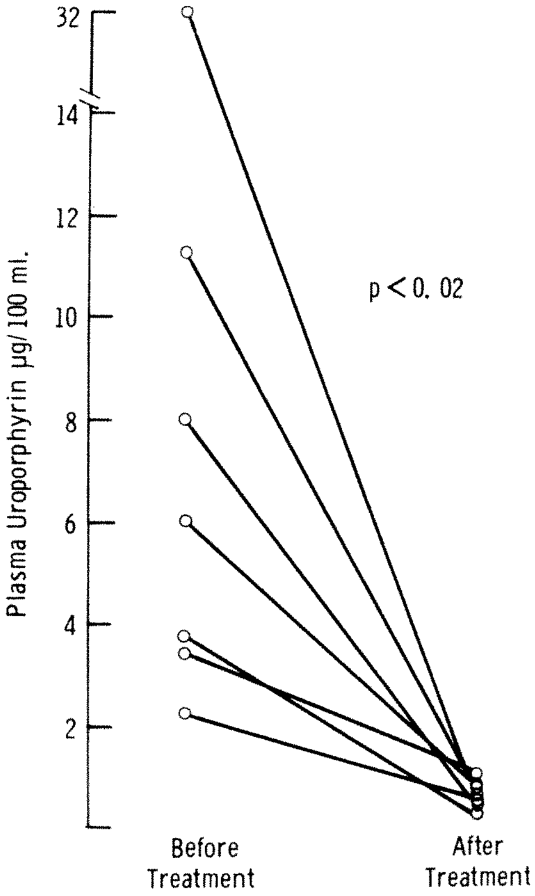

FIGURE 2. Plasma uroporphyrin in seven patients before and after treatment.

The figures from E.N. were not used in this calculation since she was known to be unable to make an accurate 24 h urine collection.

DISCUSSION

The results suggest that, in patients undergoing treatment, plasma uroporphyrin levels reflect the degree of activity of CHP. In any laboratory where porphyrins can be measured quantitatively their measurement in plasma offers no additional complications. Twenty millilitres of plasma or serum are required but as venesection is the commonest and most effective form of treatment (Ramsay *et al.*, 1974) the taking of large samples of blood is not detrimental and, during therapy, plasma can be obtained from the blood removed.

Patients undergoing plasmapheresis were participants in a pilot study in which it was not found possible to demonstrate any clear benefit. Remission in these patients is assumed to have occurred because of alcohol abstention.

At present we have insufficient data to predict recurrences. Long term studies will be necessary to say what factors are required to produce a relapse. The minimal plasma uroporphyrin level at the time of relapse and the duration of any rise are two of the factors that might be measured. Two of the patients reported have relapsed. J.C. developed slight skin fragility, with a plasma uroporphyrin of 3·5 μg/100 ml 12 months after stopping treatment. W.D. developed skin fragility again 16 months after stopping treatment. At this time his plasma uroporphyrin was 139·4 μg/100 ml. Chromatography confirmed that the substance giving this very high result was all uroporphyrin. One totally asymptomatic patient, picked up during routine urine analysis, had a plasma uroporphyrin of 9·6 μg/100 ml. Two months later it had risen to 38·4 μg/100 ml. He is at present being examined regularly for any overt signs of disease.

Day to day fluctuation has been found in urinary uroporphyrin in CHP (Copeman, Cripps & Summerly, 1966). Our own studies (unpublished) indicate that there is very little day to day variation in plasma uroporphyrin—even after provocation with alcohol. This is supported by the figures reported here which, with a few exceptions, show steady trends.

It appears therefore that measurement of plasma uroporphyrin is a most convenient and reliable method of monitoring response to treatment. The highly significant correlation between plasma and urinary levels of uroporphyrin supports this view. Occasional estimations during the course of therapy render other porphyrin analyses unnecessary although full blood, urinary and faecal analyses should always be performed initially to ensure that the diagnosis is correct. Treatment can be discontinued when the plasma uroporphyrin drops below 1·0 μg/100 ml.

ACKNOWLEDGMENT

We are grateful to Professor A. Goldberg for advice given in the preparation of this paper.

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British Journal of Dermatology (1975) **93**, 43.

Repeated tissue sampling with a dental broach

A TRIAL IN CUTANEOUS LEISHMANIASIS

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Accepted for publication 18 October 1974

SUMMARY

A simple and almost painless technique for repeated sampling of dermal infiltrates with a dendritic broach was used in the diagnosis of cutaneous leishmaniasis. This technique can be used to advantage to obtain organisms and cells underneath crusted lesions and to evaluate not only the number of organisms but also the cell pattern at different depths of the lesion. It can be used repeatedly on the same lesions without appreciably disturbing the natural progress of the disease and is therefore well suited to monitor the *in vivo* effects of therapeutic agents on organisms. Its use in twenty-one cases of cutaneous leishmaniasis and one case of cutaneous leprosy is described.

METHOD

This sampling technique is performed with a commercially available dendritic broach, which is used by dentists to ream the endodontic canal of contaminated material in the treatment of pulpitis. The needle is available in different lengths and sizes from dental supply houses. The end is either barbed (Fig. 1) or threaded. Unbarbed smooth broaches are also available. The broach is heat sterilized by flaming. The sterile needle is then pushed into the side of a lesion underneath the crust to the desired depth. No local anaesthetic is required. The material is obtained by slowly rotating the needle before withdrawal (Fig. 2). The barbs remove tissue particles, cells and organisms and the specimens are less contaminated with surface squamous epithelium and debris than with other techniques commonly used. The material is washed off in a drop of saline on a slide, smeared, air dried and then stained (Fig. 3). Our smears for leishmaniasis were stained with Giemsa stain which permitted observation of intracellular as well as extracellular organisms and of the cell types in the exudate (Fig. 4).

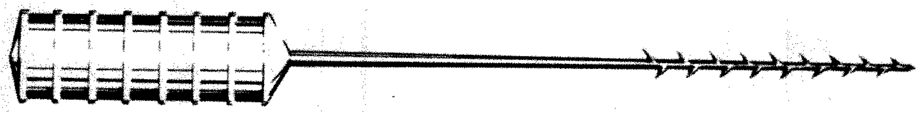

FIGURE 1. Dendritic broach.

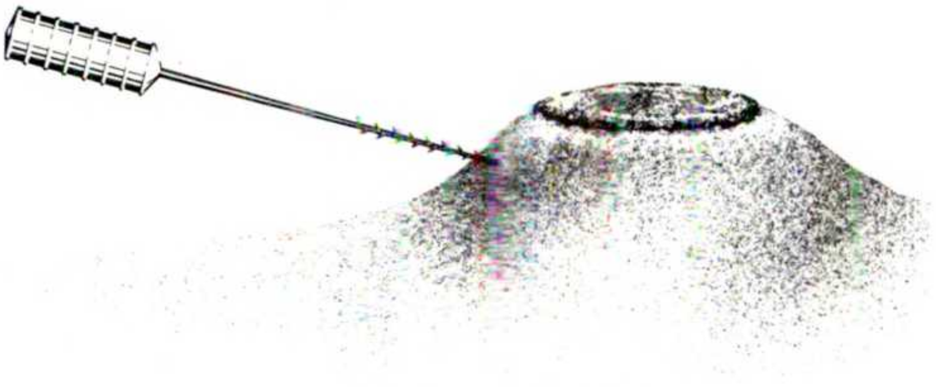

FIGURE 2. Method of obtaining specimen.

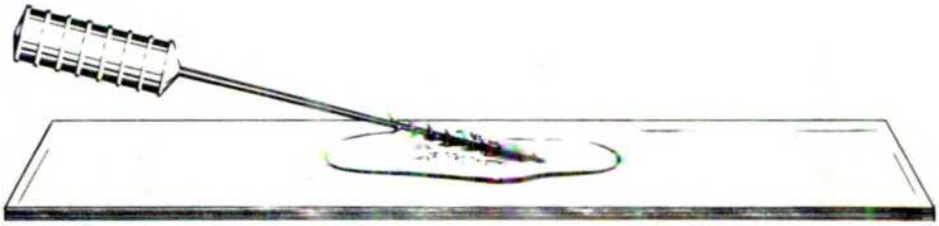

FIGURE 3. Preparation of specimen.

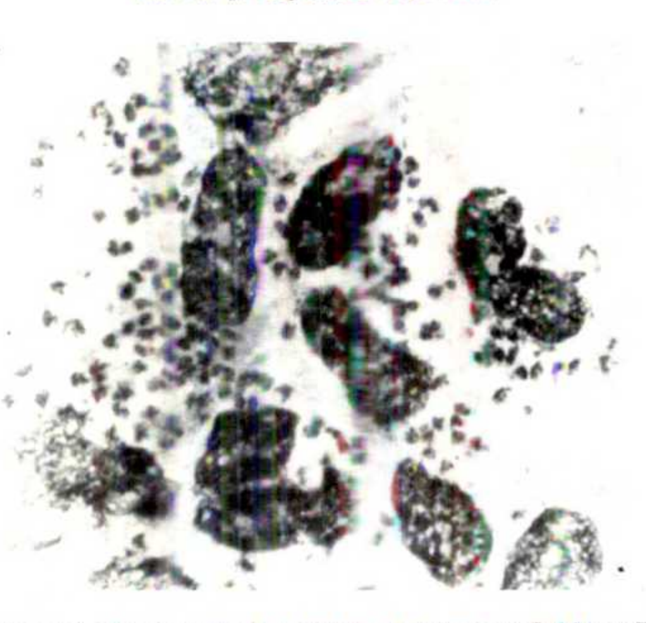

FIGURE 4. Specimen taken from acute leishmaniasis showing numerous Leishman-Donovan bodies. Giemsa stain. Oil immersion.

In one case of lepromatous leprosy, the needle was inserted into a lesion after surface sterilization and the material stained with a modified Ziehl Neelsen stain for acid fast bacilli.

A widely used method for obtaining material for the diagnosis of cutaneous leishmaniasis consists of cutting into the lesion with a scalpel and scraping the wound margin to collect cells from the dermal infiltrate.

RESULTS

A total of twenty-one lesions of cutaneous leishmaniasis in various stages of development were examined, using the technique described. The only information provided for the pathologist was the code number of the case. Seventeen lesions had been present for less than 10 months and all showed *Leishmania tropica* organisms in the smear. The number of parasites correlated well with the stage of the lesion. Fewer organisms were found in the lesions with early tuberculoid changes or central scarring with a progressing edge. Large numbers of intra- and extracellular organisms were found in fresh lesions. Four lesions which had been present for more than 10 months showed no organisms. One of these revealed a few Leishmania organisms on repeat testing. There were no false positive results in the series as only lesions which were clinically strongly suggestive of cutaneous leishmaniasis were examined in this way.

One case of lepromatous leprosy was also investigated by biopsy with the endodontic broach rather than using the scalpel skin-slit method. Organisms were obtained easily, with less trauma, less pain to the patient and less bleeding than would have been inherent in the skin-slit methods.

DISCUSSION

This method was first described by Gremliza (1956) who used it extensively in the diagnosis of skin lesions in which the causative organisms are deep to the surface. This method can be useful in monitoring the progress of the lesion under therapy, since the minimal trauma is well tolerated by the patients (who permit frequent repetition of the procedure). This method could, therefore, be assessed further for monitoring the morphological index in *Mycobacterium leprae* infections during treatment, avoiding the repeated slitting of the skin with a scalpel blade. It is equally useful to obtain material for bacteriological cultures from deep skin lesions.

The advantages of this method are its easy performance, the low price and easy availability of the needles, their repeated use after proper sterilization, the rapidity of the method, the minimal trauma to the patient and the inherent reduction of pain and bleeding as compared to the skin-slit method. A suitable and representative specimen is obtained practically every time.

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British Journal of Dermatology (1975) **93**, 47.

The percutaneous penetration of nandrolone decanoate*

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Accepted for publication 21 October 1974

SUMMARY

A study has been carried out of the penetration of nandrolone decanoate through human cadaver skin *in vitro* using radioactive tracer material. The radiochemical purity of the material desorbed from the dermal skin surface is very much less than that of the material absorbed into the epidermal surface. These observations are a consequence of the low level of overall penetration of the skin by the steroid. There is also a possibility that a small percentage of the steroid is being metabolized by micro-organisms present in the skin samples.

There has been in recent years a continuing interest in corticosteroid diffusion processes in skin (Wurster, 1972; Polano *et al.*, 1972). One technique frequently employed in work of this kind involves the use of radioactive tracer material, the penetration being studied either in terms of the total permeation of material across a skin membrane into a suitable solvent 'sink', or as the concentration profile of the radioactive species throughout the skin sample (Munro & Wilson, 1969). Whilst it is known that there is sufficient enzyme activity in freshly excised human or animal skin to effect metabolism of certain steroids, hydrocortisone being a notable example (Hsia, Musallem & Witten, 1965; Hsia & Hao, 1966), it is generally assumed in studies of the present type that cadaver skin is effectively inert, and that the steroid of interest will therefore pass unchanged through the sample. Rarely is the radiochemical purity of the tracer material monitored throughout the diffusion process. The need for such a check is clearly apparent in the present study involving the percutaneous migration of nandrolone decanoate-4-^{14}C (hereafter abbreviated to ND-^{14}C), where the radiochemical purity of the tracer appears to be progressively degraded as the penetration proceeds.

MATERIALS AND METHODS

Skin samples

Human cadaver abdominal skin was obtained from eight females and seven males in the 50–70 year age group. Sheets of dermis were prepared from it by removal of the epidermis following the procedure described by Polano *et al.* (1972). Removal of the stratum corneum was effected by stripping samples of whole skin with adhesive cellophane tape (Wolf, 1939).

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* Chemical name; 17β-hydroxy-4-estren-3-one decanoate. Proprietary name; Deca-Durabolin (Organon).

Scintillation liquids

Scintillation liquids were made up as follows. Bray's scintillator: 64 g naphthalene, 4 g 2,5-diphenyloxazole (PPO) and 200 mg 2,4-di-2-(5-phenyloxazolyl) benzene (POPOP) were dissolved in 100 ml methanol and 20 ml ethylene glycol, and the volume then made up to 1 l with 1,4-dioxan. Toluene scintillator: 8 g PPO and 200 mg POPOP were dissolved in toluene, the volume being made up to 1 l. PPO and POPOP were used as supplied by Koch-Light Laboratories Ltd., Colnbrook, Bucks, England.

Radiolabelled steroids

Nandrolone-4-^{14}-C of specific activity 31·4 mCi/mg was purchased from Fluorochem Ltd. and stored in ethanol solution of activity 3·50 μCi/ml.

Nandrolone decanoate-4-^{14}-C (ND-^{14}C) of specific activity 71·8 μCi/mg was prepared by the Radiochemical Laboratory, Organon Scientific Development Group, Oss, The Netherlands, and stored in ethanol solution having an activity of 4·68 μCi/ml.

Chromatography

Thin layer chromatography (TLC) was carried out using 2·5 mm thick silica layers (Merck 254) elutedl with benzene/ethyl acetate (7:3). The chromatograms were developed auto-radiographically with Kodirex X-ray plates exposed for 24 h at 23°C. On the basis of the distribution of the spots on the autoradiographs, the silica plates were divided into sections, the silica in each section transferred to a counting vial and the level of radioactivity estimated as described below using toluene scintillation liquid.

Penetration studies

Ten membranes of whole skin, and ten of stripped skin were each mounted in glass diffusion cells, such that diffusion would occur across a circular area of skin 1·7 cm in diameter. The lower compartment of each cell contained 5 ml of 0·9% aqueous saline solution, acting as a sink for material desorbed from the dermal skin surface. ND-^{14}C was applied as an alcohol solution to the epidermal skin surface, the solvent being immediately evaporated off under a stream of nitrogen. After a period of 18 h, during which time the cells were maintained at 30°C and 90% relative humidity, three 1·0 ml aliquots were taken from the saline sink and the total radioactive material present estimated by liquid scintillation counting using a Nuclear Chicago Unilux II Counter and Bray's scintillation liquid. The level of penetration so measured, expressed as the percentage of the applied dose desorbed into the saline sink, is quoted in Table 1.

TABLE 1. Total radioactive material desorbed from the dermal skin surface after 18 h*

Whole skin	0·006 ± 0·001
Stripped skin	0·6 ± 0·1

* Expressed as a percentage of the applied dose; mean ± s.e. of 10 determinations.

The radiochemical purity of the desorbed material was estimated in a subsequent experiment in which fourteen sections of stripped skin and fourteen sections of dermis were each mounted in a diffusion cell, 0·2 ml of ethanolic ND-^{14}C solution were then applied to the epidermal face of each section, and the ethanol evaporated off immediately under a stream of nitrogen. The cells were maintained for 18 h at 27°C and a relative humidity of 90%. At the end of this period the saline was extracted with methylene chloride, the extracts centrifuged, concentrated to a suitable volume by evaporation under nitrogen, and applied to previously prepared TLC plates.

Finally, a further ten samples of whole skin, and ten of stripped skin, were each mounted in a diffusion cell, ND-^{14}C was applied to the epidermal surfaces, and the diffusion allowed to proceed exactly as above. After a period of 18 h each membrane was cut into 25 μm sections in a direction parallel to the skin surface using a Leitz 1310 freezing microtome, the sections were dissolved in aqueous alkali and counted as before using Bray's scintillator. Prior to counting, aliquots of the alkali solution were taken and the protein content estimated by the method of Lowry *et al.* (1951).

Incubation experiments

Whole human cadaver skin (2 g) was homogenized in 20 ml of freshly prepared Krebs–Ringer solution. Three 2·0 ml aliquots were each then incubated at 27°C for 20 h, and a further three for 24 h, with 25 μl of ethanolic ND-^{14}C solution. The entire experiment was then repeated using Krebs–Ringer solution containing 300 mg benzylpenicillin (sodium) B.P. and 500 mg streptomycin to each 100 ml of solution. At the end of the specified period each mixture was extracted with methylene chloride, the extracts centrifuged, concentrated to a suitable volume, and applied to the TLC plates as before. Typically, the efficiency of recovery of the radioactive material by this method is of the order of 50%.

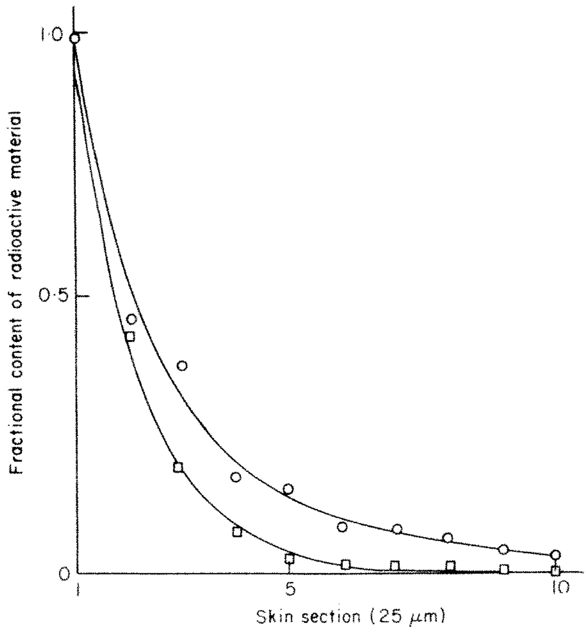

FIGURE 1. Profile of radioactivity through whole human cadaver skin (□) and stripped human cadaver skin (○) after epidermal application of ND-^{14}C.

RESULTS AND DISCUSSION

In Table 1, the total quantity of radioactive material penetrating into the saline sink is shown. It is immediately apparent that only a very small fraction of the applied material is able to penetrate through the skin membrane. Although the level of penetration is significantly increased by removal of the stratum corneum, in absolute terms the fractional penetration is still exceedingly low.

In Fig. 1 the average distribution profile of radioactive material throughout ten stripped and whole skin membranes is shown. The profile is estimated as the concentration of radioactive material per mg of protein for each 25 μm section, and expressed as a fraction of the concentration observed in the first, epidermal, section. It can be seen that very little radioactivity is to be found beyond the first 250 μm, and most is held in the first 125 μm. It is also apparent that there is no very dramatic change in the profile resulting from removal of the stratum corneum; the epidermis, therefore, also presents an appreciable barrier to the penetration of ND-^{14}C.

Subsequently, a study was made of the radiochemical purity of the material desorbed from the dermal face of sections of stripped skin and from dermis alone, dermis being used in place of whole skin since this provides a much higher level of penetration without otherwise affecting the conclusions of the experiment. An 'average' chromatogram of the material desorbed into the saline sink from the two types of membrane is presented diagrammatically in Fig. 2, from which it is immediately clear that less than 10% of the radioactivity present in the sink is attributable to ND-^{14}C, whilst some 70–80% is due to some other, single species. The identity of this material is not completely certain;

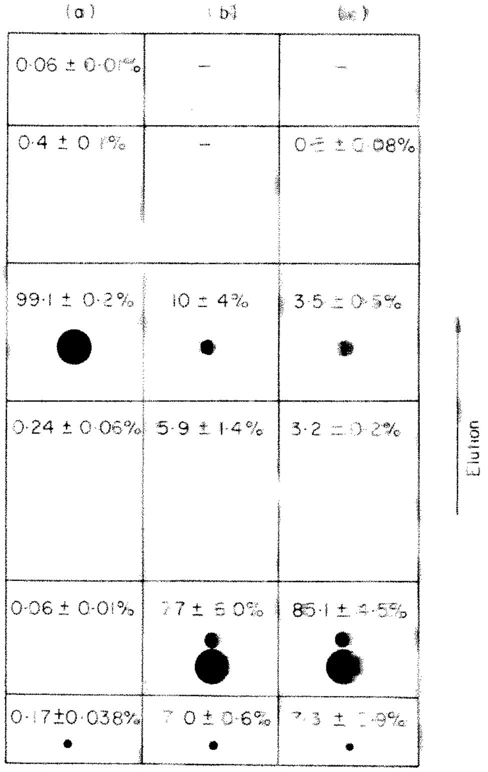

FIGURE 2. TLC of ND-^{14}C: (a) alcohol solution before application to skin, (b) material desorbed from stripped skin, and (c) material desorbed from dermis only. Quoted figures give the percentage radioactive material found within each area of the plates.

it cannot, however, be separated from an authentic sample of nandrolone-4-^{14}C by the TLC system employed here, and the probability is that the material is in fact nandrolone-4-^{14}C.

Two explanations for the above effect have been considered. First, although the enzyme activity of skin is rapidly lost, and is unlikely to persist in cadaver skin of the type studied here, nevertheless a degree of metabolism of the steroid might be brought about by micro-organisms indigenous to skin, which might conceivably proliferate after excision (Polano *et al.*, 1972). To check this point, samples of ND-^{14}C have been incubated with human cadaver skin in the presence and absence of antibiotic agents. TLC of the methylene chloride extract of the mixtures after incubation indicated a material having similar R_F value to the 'unknown' observed in the skin penetration experiments. The percentage ND-^{14}C and 'unknown' are quoted in Table 2. Clearly, the conversion of ND-^{14}C is slight, but none the less is significant. Furthermore, the reduced conversion observed in the presence of antibiotics is also felt to be significant, and lends support to the view that indigenous micro-organisms may be responsible, in part, for the effect. The overall conversion is, however, too small to be wholly responsible.

TABLE 2. Percentage ND-^{14}C and 'unknown' in the material resulting from incubation of ND-^{14}C with whole human cadaver skin in Krebs–Ringer solution at 27°C

	ND-^{14}C (%)	'Unknown' (%)
(a) Ethanol solution of ND-^{14}C	98·4±0·4	0·11±0·02
(b) Incubated with Krebs–Ringer, 24 h	97·6±0·3	0·90±0·10
(c) Incubated with skin homogenate, 20 h	96·1±0·7	1·96±0·06
(d) Incubated with skin homogenate, penicillin and streptomycin, 20 h	97·7±0·2	1·22±0·01
(e) As (c), 24 h	96·9±0·1	2·2±0·04
(f) As (d), 24 h	97·1±0·2	1·5±0·03

A second explanation which has been considered is that the skin membrane is effecting something akin to a chromatographic separation. From Fig. 1 and Table 1 it is apparent that most of the radioactive material is to be found in the first 200 μm of the skin, and that the fraction of the applied dose which is desorbed from the dermal face is minute. Under these conditions, a quite small percentage impurity in the ND-^{14}C stock solution might conceivably appear as an appreciable proportion of the material desorbed from the skin, provided such material had a higher penetration rate than ND-^{14}C. Such an effect would be further enhanced by a quite small enzymic conversion occurring as the penetration proceeds. It is interesting to note that nandrolone-4-^{14}C has a very much higher rate of penetration than ND-^{14}C; under the same experimental conditions as described above, 2·1±0·5% and 28±3% of the applied dose of nandrolone-4-^{14}C is desorbed into the saline sink after penetration through whole skin and stripped skin respectively.

Whilst more detailed study is required to identify more clearly the source of the observed effect, nevertheless the present results seem to be of general importance in the context of skin penetration studies involving radioactive tracer material. It is true that a steroid ester, such as that considered here, is likely to be more prone to enzymic conversion than other molecules, for which the possibility of metabolism during the diffusion process might reasonably be disregarded. However, the important point arising from the present study is that, whenever the penetration rate is very low, as it frequently is for steroid molecules, small radioactive impurities having a faster penetration rate than the material of interest will appear as a proportionately higher percentage of the tracer as the diffusion proceeds. It is felt therefore that studies of this kind should include a check of the radiochemical purity of the tracer throughout the process.

ACKNOWLEDGMENT

The authors are grateful to Miss Fiona Talbot for technical assistance.

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Secondary syphilis: a clinico-pathological review

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Accepted for publication 21 October 1974

SUMMARY

The histological appearances found in biopsies from fifty-seven patients with secondary syphilis have been correlated with the clinical morphology of the eruptions. Considerable variation of histological pattern was encountered, and the frequency with which some of the classically described changes were found to be absent or inconspicuous is stressed. Of particular interest were the findings that, in nearly one-quarter of the biopsies, plasma cell infiltration was either absent or very sparse, and that vascular damage was seen in less than half. Where present, the vessel changes were almost entirely confined to swelling of the endothelial cells. Proliferation of the endothelial cells was most uncommon.

The epidermis was very frequently involved in the inflammatory process. Exocytosis, spongiosis, parakeratosis, and acanthosis were the most frequent changes. No consistent histological difference between papular and papulo-squamous lesions could be found but macular lesions demonstrated more superficial and less intense dermal infiltration as well as less severe epidermal involvement.

In late secondary lesions, the infiltrate became granulomatous, but in other respects the duration of the exanthem could not be correlated with the pathology.

The differential diagnosis from pityriasis lichenoides and other inflammatory dermatoses is discussed and the value of histopathology in the diagnosis of secondary syphilis is emphasized.

An increasing incidence of early infectious syphilis has been recognized in many parts of the world over the last 20 years (Willcox, 1972). In Great Britain, despite a decline in the number of new cases in recent years (Report of the Chief Medical Officer, 1970) it continues to be prevalent, and particularly amongst male homosexuals (Waugh, 1972).

Over these last two decades, the number of cases of secondary syphilis presenting at St John's Hospital has doubled, and the number of biopsies examined has increased by the same proportion. This accumulated clinical and histological material forms the basis of this report.

The histopathology of secondary syphilis has usually been described in relationship to the clinical morphology of the eruption (Lever, 1967; Montogomery, 1967; Johnson, 1972; Grosshans, 1973). It was our impression from previous experience, that this relationship was imprecise. In order to examine this clinico-histological relationship, sections were examined and recorded in the absence of the clinical details and correlated subsequently.

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MATERIALS AND METHODS

Biopsy specimens of fifty-seven serologically confirmed cases of secondary syphilis have been seen at St John's since 1952. Six patients had more than one biopsy performed, making a total of sixty-four biopsies. With the exception of six specimens all biopsies had been taken from the trunk or limbs. The remaining specimens consisted of two palmar, one plantar, two scalp and one facial lesion. The patients' ages ranged from 17–59 years, with more than half being in their third decade. The male to female ratio was 3·7:1. The rashes had been present for between 1 week and 1 year. In one case the eruption had recurred over a 3-year period.

As ascertained from the clincal records and photographs, twenty-nine of the lesions were classified as papules, twenty as papulo-squamous, and thirteen as macular lesions. In two patients the clinical data were insufficient for adequate classification.

Haematoxylin and eosin sections were examined in all cases and in fourteen the following additional stains were used: Periodic acid Schiff, orcein elastic, Gomori's reticulin, Perle's blood pigment and Masson-Fontana melanin.

The various pathological changes were recorded on a standard proforma, conforming to the headings

TABLE 1. Histological features of sixty-four biopsies of secondary syphilis and the sixty-two with known morphology

	All lesions				
	Number	(%)	Macules	Papules	Papulo-squamuos
No. of biopsies	64	100	13	29	20
Surface change	6	9	—	3	3
Hyperkeratosis	12	19	—	7	5
Parakeratosis	26	41	1	14	11
Acanthosis	20	31	1	11	8
Spongiosis	40	62·5	4	23	13
Keratinocyte necrosis	19	30	3	11	5
Exocytosis					
(i) all	54	84	7	28	18
(ii) mononuclear	36	55	6	18	11
(iii) mixed	18	28	1	10	7
microabscess spongiform pustulation	13	20	—	8	5
Papillary oedema	34	53	2	19	13
Papillary RBC extravasation	17	23	1	9	5
Vascular thickening	27	42	5	10	12
Inflammatory cell infiltration	64				
(i) sparse	6	9	4	2	—
(ii) moderate	23	36	5	9	9
(iii) dense	33	52	4	18	11
Plasma cells absent or very sparse	15	23	3	9	3

of Table 1. Only the presence or absence of each feature was recorded together with a qualifying descriptive comment. The dermal inflammatory infiltrate was scored by an arbitrary three plus system corresponding to sparse, moderate, or dense. The infiltrating cells were also characterized morphologically. At least two observers scored the lesions independently and the recorded data represented an amalgam of these observations.

In eleven of the cases, the correct diagnosis was not suspected initially, and in nine, syphilis was only considered as a possibility in the differential diagnosis. In these twenty cases the clinical diagnoses most commonly made were in order of frequency—pityriasis lichenoides, psoriasis, eczema, insect bites, sarcoid, leishmaniasis and lymphoma.

Histopathology

(a) *The epidermis* (Table 1). The degree to which the epidermis was involved varied considerably. Nearly one-third (20 specimens) showed mild to moderate acanthosis and in two of these biopsies striking elongation of the rete ridges gave a psoriasiform outline to the epidermal contour (Fig. 1). In the remainder, the epidermis appeared either unchanged or attenuated. This thinning of the epidermis was typically seen in lesions in which intense papillary oedema was also present (Fig. 2). Epidermal oedema of mild to severe extent characterized nearly two thirds (40) of the specimens but its intensity did not correlate directly with the intensity of the dermal reaction below.

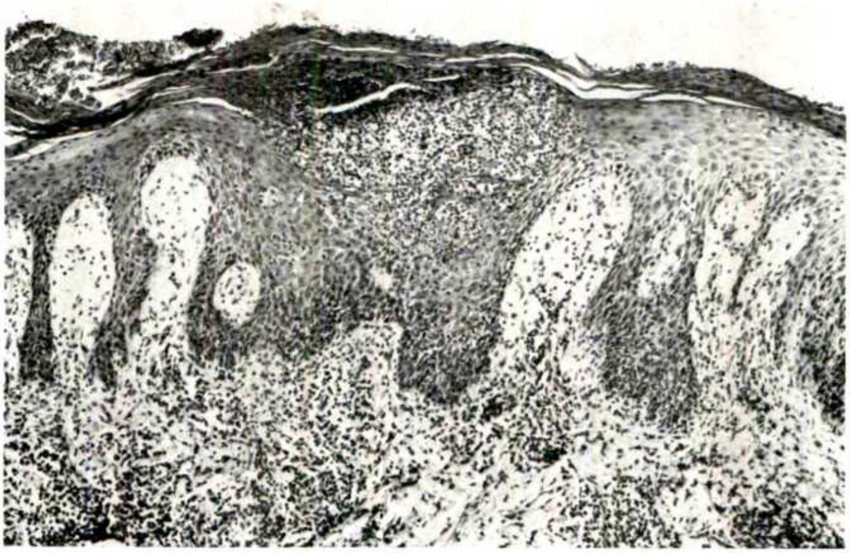

FIGURE 1. Papulo-squamous lesion (4 weeks) showing psoriasiform epidermal hyperplasia and spongiform pustulation. The papillae are markedly oedematous and the infiltrate is predominantly disposed around dilated vessels. (H & E × 100.)

Exocytosis (defined as the presence of inflammatory cells in the epidermis) was the most constant feature (53) and was of two distinct types. In thirty-five, this infiltrate was composed of small round mononuclear cells only, occurring in the epidermis diffusely or in small foci. In eighteen sections, both polymorphonuclear and small round cells had invaded the epidermis and in thirteen of these, aggregations had occurred producing collections of cells in the stratum corneum resembling Munro abscesses (Fig. 3) and less frequently spongiform pustulation (Fig. 1).

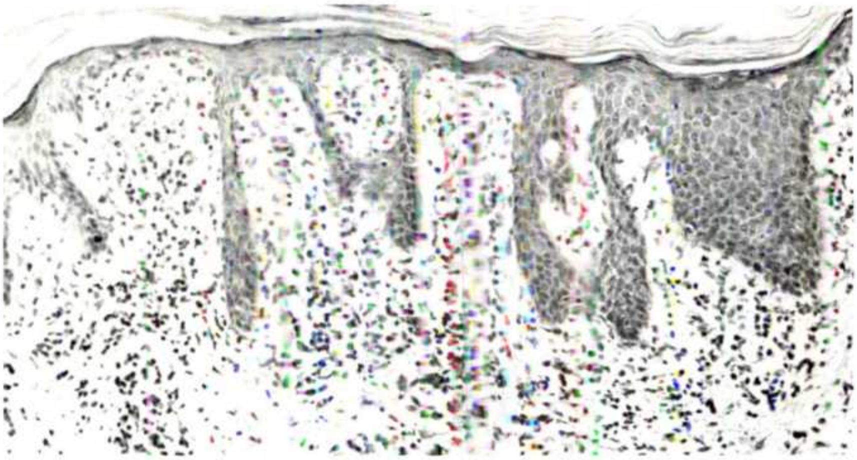

FIGURE 2. Papulo-squamous lesion (6 weeks). An irregularly acanthotic epidermis is thinned over oedematous papillae. Note the pronounced plasma cell infiltration of the dermis. (H & E × 100.)

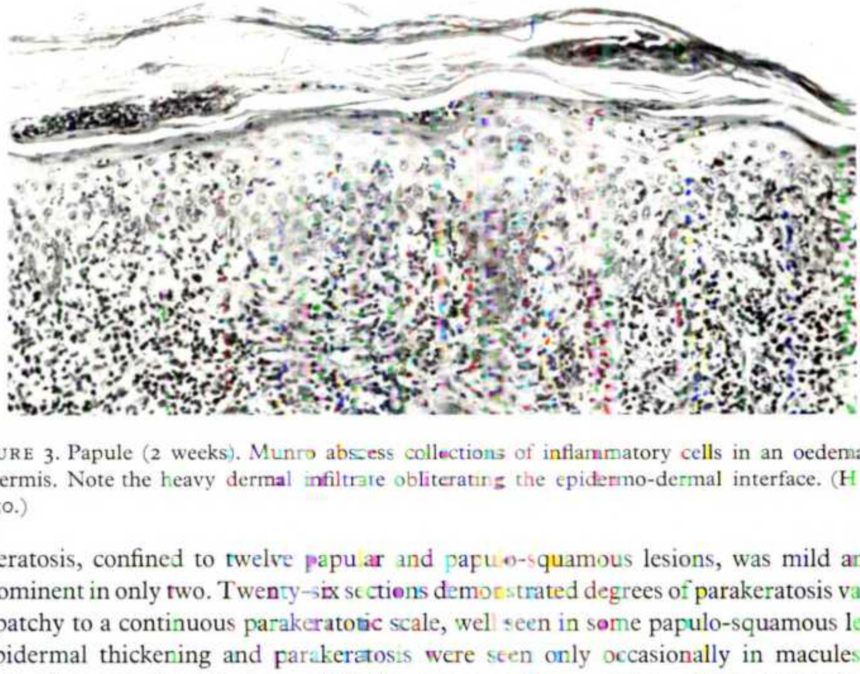

FIGURE 3. Papule (2 weeks). Munro abscess collections of inflammatory cells in an oedematous epidermis. Note the heavy dermal infiltrate obliterating the epidermo-dermal interface. (H & E × 150.)

Hyperkeratosis, confined to twelve papular and papulo-squamous lesions, was mild and focal in ten and prominent in only two. Twenty-six sections demonstrated degrees of parakeratosis varying from mild and patchy to a continuous parakeratotic scale, well seen in some papulo-squamous lesions.

Mild epidermal thickening and parakeratosis were seen only occasionally in macules, although more than half (seven) showed some mild inflammatory cell permeation of the epidermis.

Occasionally (19), single or small groups of epidermal cells were encountered undergoing hyaline necrosis (Fig. 4). In four specimens partial erosion of the upper epidermis with crusting was noticed, but no example of complete ulceration of the Lues Maligna type was seen.

(b) *The dermis* (Table 1). The inflammatory infiltrate was always most intense in the papillary dermis but generally the infiltrate also extended perivascularly into the reticular dermis. It did not tightly sleeve the vessels as in a figurate erythema, but was loosely dispersed to involve the surrounding tissue. In only four biopsies, all from macules, was the infiltrate limited to the upper dermis (Fig. 5).

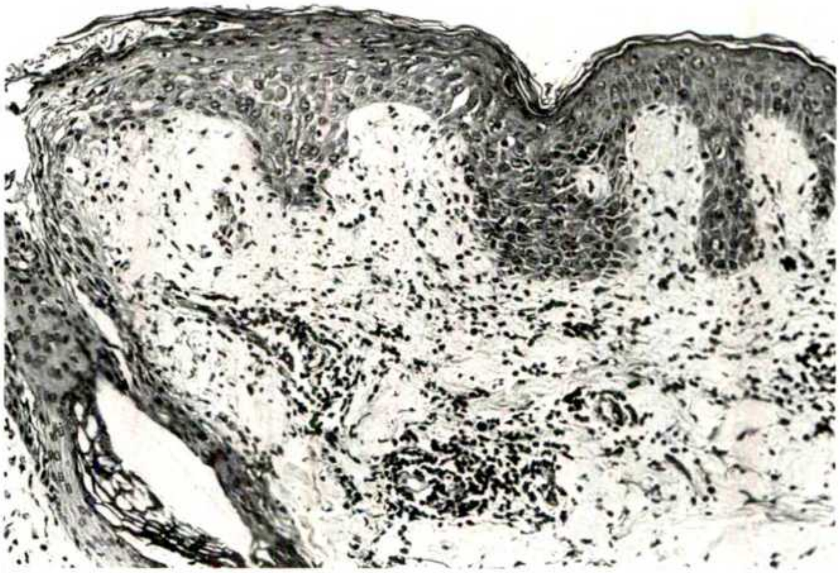

FIGURE 4. Papule (1 week). Focal epidermal parakeratosis and spongiosis with a group of necrotic keratinocytes (hyaline bodies). (H & E × 100.)

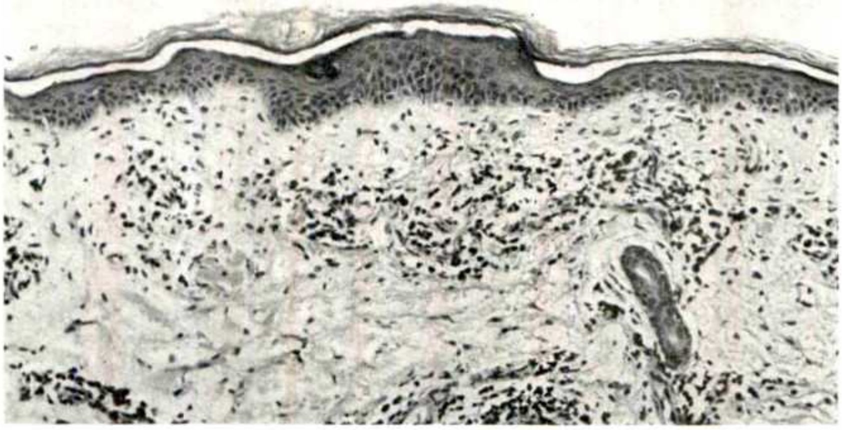

FIGURE 5. Macule (8 weeks). A loose lymphocytic infiltrate surrounds the superficial dilated vessels. Plasma cells are prominent in this case. (H & E × 60.)

Almost all papular and papulo-squamous lesions (47 of 49) demonstrated a moderate or dense inflammatory cell infiltrate which in many sections obliterated the overall perivascular pattern (Fig. 3), producing a lichenoid appearance in the upper dermis. In predominantly granulomatous lesions confluent areas of infiltrate also extended into the deeper dermis. In the larger biopsies, this perivascular arrangement could be seen to extend deeply around a single or a small number of deep dermal vessels. The follicles and sweat glands were very frequently sleeved by inflammatory cells and in eight biopsies the focal point of the reaction was centered around the pilosebaceous units giving the appear-

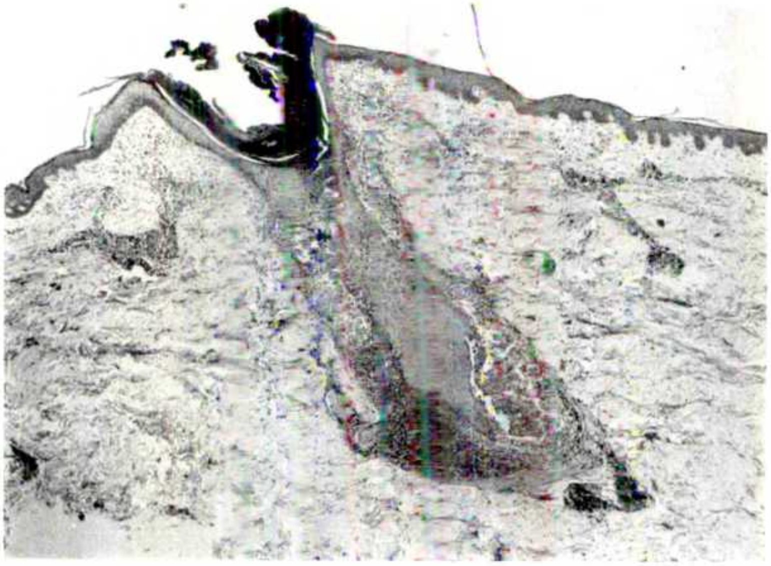

FIGURE 6. Follicular papule (8 weeks). Note the destructive follicular inflammatory reaction with some sleeving of adjacent vessels. (H & E × 40.)

ances of a folliculitis (Fig. 6). Although the follicular epithelium showed exocytosis and parakeratotic horny plugging, destruction of the follicle was seen in only one biopsy. In two specimens, the infiltrate occupied the perineural space, but in none were the nerves themselves infiltrated.

For descriptive purposes the infiltration was classified as being (a) predominantly lymphocytic (3); (b) lympho-histiocytic (43); (c) predominantly histiocytic (9); and (d) predominantly granulomatous (9). Plasma cell infiltration occurred in varying degree with each category. The density of this plasma cell infiltrate could not be correlated with either the stage of the disease or the types of other cells present.

Large pale histiocytes, aggregations of epithelioid cells and multinuclear giant cells were seen in two lesions of 9 weeks duration and a granulomatous infiltrate characterized all lesions of 16 weeks duration or more (Fig. 7) with a single exception. In this instance antibiotic treatment had caused partial resolution before a specific diagnosis was reached. In two specimens focal granulomas were found in lesions of 4 weeks duration only.

Severe papillary oedema accompanied the infiltrate in thirty-four biopsies and red cell extravasation also in seventeen cases. Vasodilatation was very common, but significant vessel damage was, by contrast, infrequent. In twenty-four biopsies noticeable swelling of the endothelial cells was apparent but usually not prominent. Endothelial proliferation was seen in only three cases, and in two of these the eruption had been present for 26 and 52 weeks respectively.

In six patients multiple biopsies had been performed. The histological patterns differed only in those two cases in which different morphological lesions had been sampled.

Little additional information was obtained from the histochemical stains. Some papules showed loss of elastic tissue.

DISCUSSION

The old adage that syphilis is the great mimic is perhaps just as true for the histologist as it is for the

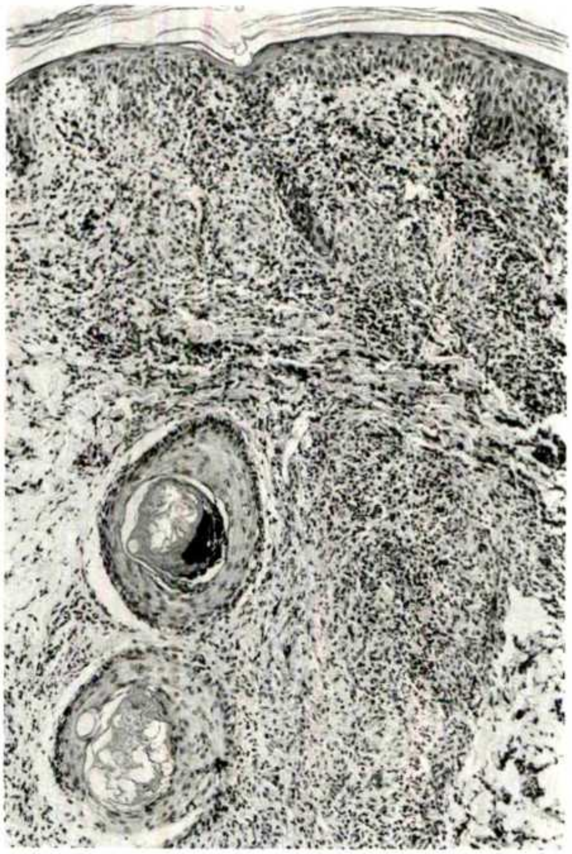

FIGURE 7. Papulo-squamous lesion (20 weeks) A diffuse granulomatous infiltrate occupies most of the dermis with cells extending into the epidermis as well. (H & E × 60.)

clinician. This study has detailed both the wide variety of histological change that occurs in secondary syphilis and its value in the diagnosis of the unsuspected case. Indeed on occasions it has been possible to alert the clinician to the possibility of unsuspected syphilis because of the histological appearances.

Certainly many biopsies conform to the classically described features of secondary syphilis. Prominent among these are the presence of plasma cells in a perivascular infiltrate extending deeply in the skin, accompanied by evidence of endothelial damage to small blood vessels. However, these features cannot be relied upon, and in particular minor degrees of endothelial damage are difficult to assess and are a common feature in many other inflammatory dermatoses. Even the presence of plasma cells is not invariable, but in their absence it may still be possible to suspect syphilis by careful consideration of the total histological picture. In our material, plasma cells were absent or inconspicuous in nearly 25% of the biopsies. Alteration of small blood vessels was usually confined to endothelial swelling and prominent endothelial proliferation was very uncommon. In only one specimen the presence in the lower dermis of swollen tortuous arterioles showing obvious endarteritis was an exceptional finding. Excluding vasodilatation, the vessels were unaffected in more than half the biopsy specimens examined.

In our material the epidermis was found to be involved in more than three-quarters of all the biopsies. While the severity and extent of this involvement varied considerably, we do not believe that the frequency with which this tissue is affected has been sufficiently emphasized in the past (Lever, 1967; Montgomery 1967).

No clear histological differences between the papular and papulo-squamous lesions were detected. However, Table 1 shows that the incidence of epidermal involvement in macular lesions was less. There was also less dense inflammatory infiltration in the macular lesions, and it tended to be more superficially orientated. In many macular lesions, the histology was often not distinctive, particularly if plasma cells were few in number and biopsy may be of little practical help in the diagnosis of some of these cases.

Differential diagnosis

In sections demonstrating a perivascular infiltrate as well as epidermal involvement, eczema, psoriasis, lichenoid reactions, erythema multiforme, and drug eruptions must be considered in the differential diagnosis of secondary syphilis. However, the presence of infiltrate at much deeper levels in the dermis in syphilis should normally rule out the essentially superficial reactions found in eczema, psoriasis, and the lichenoid conditions. The characteristic histological changes of erythema multiforme are focused upon the epidermo-dermal interface, where oedema and epidermal cell vacuolation lead to necrosis and subepidermal vesicle and bulla formation (Ackerman, Penneys & Clark, 1971). The eosinophilia frequently accompanying drug eruptions is a feature not seen in secondary syphilis.

The presence of a heavy upper dermal infiltrate with invasion of mononuclear cells into the epidermis may simulate a premycosis or mycosis fungoides. In mycosis fungoides, however, large hyperchromatic mononuclear cells with crenated nuclear outline may be seen especially among these cells colonizing the epidermis. In syphilis, particularly in papular lesions, the infiltrate tends to take on a histiocytic or granulomatous quality which is usually lacking in mycosis fungoides. Large hyperchromatic mononuclear cells were seen in only one biopsy, in which they represented an extremely small proportion of the lympho-histiocytic infiltrate which also contained abundant plasma cells.

Pityriasis lichenoides is frequently the most difficult condition to exclude both clinically and histologically. Not infrequently, papular lesions were encountered showing focal epidermal changes of oedema, exocytosis, parakeratosis and keratinocyte necrosis overlying oedematous inflamed papillae with capillary haemorrhage. The syphilitic reaction pattern may closely resemble the changes of pityriasis lichenoides especially where plasma cells are absent. This pityriasis lichenoides-like reaction requires particular emphasis as this was also the most frequent clinical mis-diagnosis. However, deep perivascular infiltration is usually seen in pityriasis lichenoides only in association with acute necrotic lesions.

The formation of granulomatous foci in the dermis was the sole feature which appeared to correlate with the duration of the eruption. In eruptions of 2–4 months' duration granulomatous foci were common, and in older untreated lesions they were consistently present.

While no attempt has been made to classify each individual reaction pattern, the report by Jeerapaet & Ackerman (1973) also produced similar conclusions. They emphasized the epidermal involvement, particularly the exocytosis, spongiform pustulation, and keratinocyte necrosis. They also found a similar proportion of cases without plasma cell infiltration but quoted a much higher proportion of biopsies showing changes in the blood vessels.

Johnson's review (1972) of forty-seven cases of secondary syphilis and the description by Pinkus & Mehregan (1969) both described the epidermal involvement and the relative paucity of plasma cells. Neither, however, gave the order of frequency with which these situations may arise.

The characteristic features of secondary syphilis seen in this study were those of a deeply extending perivascular inflammatory infiltrate containing a variable proportion of plasma cells. This infiltrate was mainly lymphohistiocytic but became frankly granulomatous in the older lesions. The dermal blood vessels were usually dilated but thickened endothelial cells were present in less than half of the specimens. Papillary oedema and erythrocyte extravasation sometimes accompanied these changes.

The epidermis was very frequently involved, particularly in the papular lesions. Exocytosis, sometimes in the form of Munro abscess or spongiform pustulation, was the commonest reaction and was almost always associated with epidermal oedema. Parakeratosis, acanthosis and keratinocyte necrosis were less commonly present.

ACKNOWLEDGMENTS

We wish to acknowledge the help of Mr E.Wheeler and Mr S.Robertson and their staff in preparation of the sections and photomicrographs for this work.

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Rosacea of common male baldness

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Accepted for publication 29 October 1974

SUMMARY

Four patients are described in whom rosacea occurred on the face, and on the scalp in patches of baldness. Histopathological examination of the specimens taken from the scalp showed changes of rosacea and basophilic degeneration of collagen. In two patients direct immunofluorescence studies were performed; in one of these the presence of IgG deposits at the dermo-epidermal junction was demonstrated.

These observations confirm the influence of external factors in the pathogenesis of rosacea.

Marks & Wilson Jones (1969) described disseminated rosacea in fourteen patients in whom papular lesions on the limbs developed, similar in appearance to those on the face.

Röckl, Schröpl & Scherer (1969) reported the co-existence of rosacea lesions on the face and over the sternum in two cases. The authors reviewed textbooks of dermatology, which described the disease on the lateral surface of the neck, the back of the neck, the trunk, the limbs and the scalp. It seems that there are no reports of rosacea on the scalp in areas of baldness.

CASE REPORTS

Four patients with male pattern baldness and rosacea lesions on the face and on the scalp were examined. In patches of baldness on the parietal, frontal and occipital areas, erythema, numerous telangiectases, papules, pustules and crusts were found. The most pronounced rosacea lesions were present in the first case (Figs. 1, 2).

The clinical data are presented in Table 1.

Histopathological examination

In the specimens taken from papules and pustules on the scalp there were oedema, basophilic degeneration of collagen and dilated blood vessels filled with erythrocytes, in the upper part of the dermis; inflammatory infiltration of lymphocytes, histiocytes, polymorphonuclear leukocytes, epithelial and plasma cells around the vessels, hair follicles and sebaceous glands; intrafollicular accumulation of neutrophils and layered keratin in a dilated lumen of the hair follicle. In the first case a heavy non-specific or tuberculoid infiltrate with giant cells was found around a hair follicle (Fig. 3).

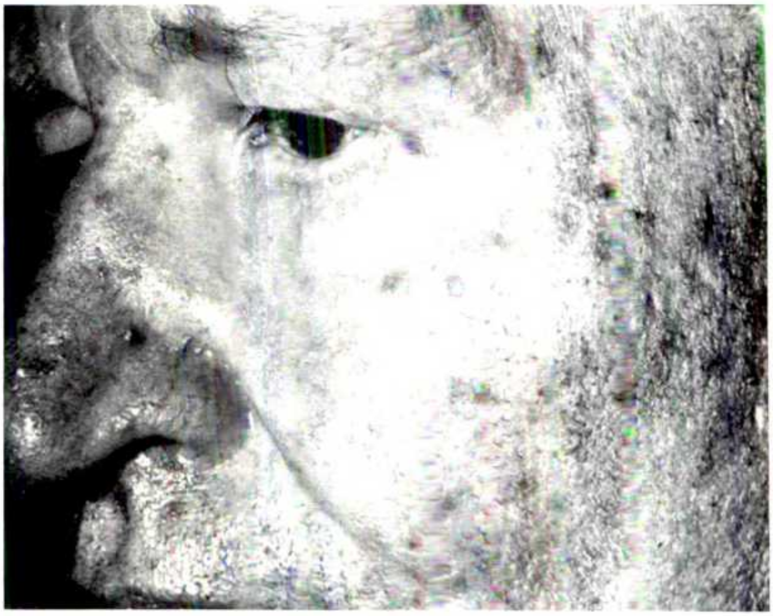

FIGURE 1. Case 1. Rosacea lesions on the face.

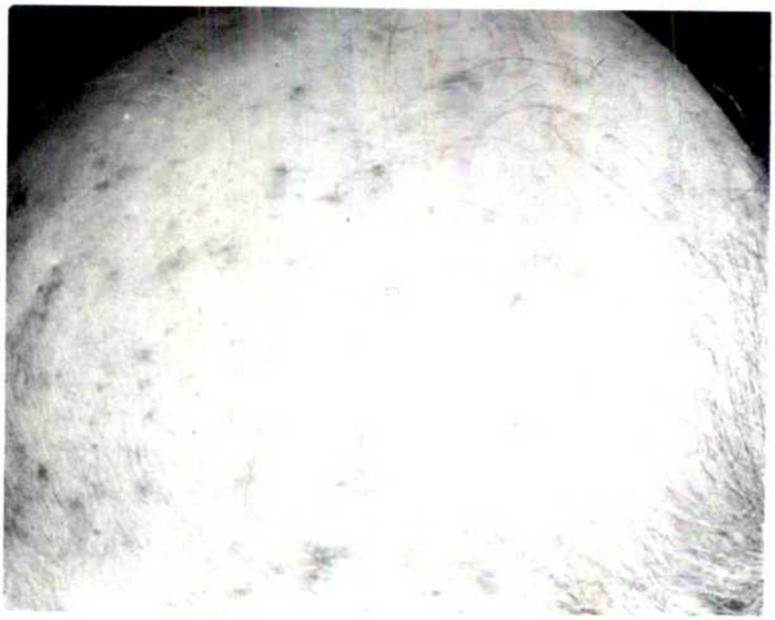

FIGURE 2. Case 1. Rosacea lesions on the scalp in patches of common male baldness.

TABLE 1. Clinical data

	Case no.			
	1	2	3	4
Age (yr)	73	67	45	64
Duration of rosacea	1 yr	9 months	2 yr	1 yr
Age of onset of baldness (yr)	40	28	28	49
Duration of baldness (yr)	32	38	15	14
Occupation	Retired bricklayer	Retired clerk	Clerk	Clerk

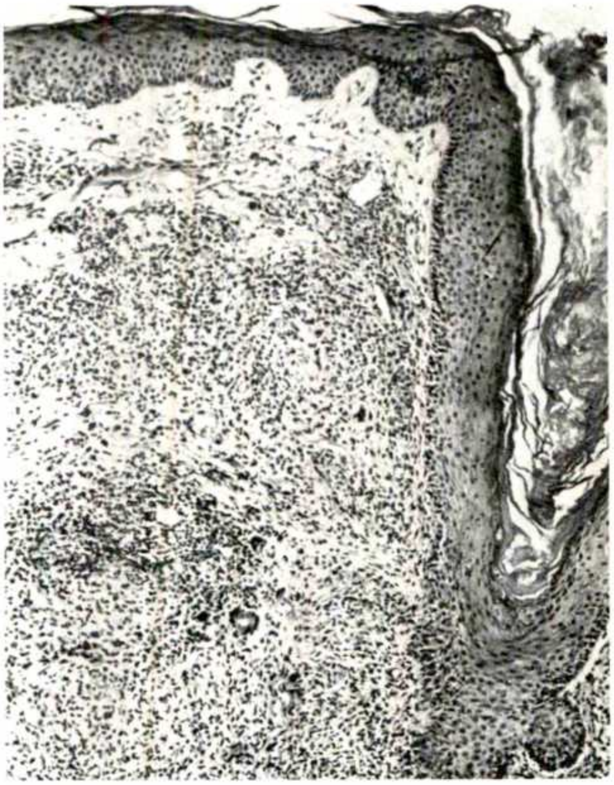

FIGURE 3. Case 1. Large tuberculoid and nonspecific infiltrate around hair follicle. Section of the specimen from the lesion on the scalp stained with haematoxylin and eosin (× 80).

Immunopathological examination

At the dermo–epidermal junction, as in lupus erythematosus, immunoglobulins have been found in rosacea and in telangiectasias of various origins by immunofluorescence studies (Jablonska, Chorzelski & Maciejowska, 1970).

In two patients receiving treatment (cases 2 and 3) biopsies of neck and telangiectatic skin from the scalp were tested by a direct fluorescent antibody technique. Deposits of IgG were detected at the dermo–epidermal junction in one (case 3).

(This investigation was carried out in the Department of Dermatology, Medical Academy in Warsaw.)

DISCUSSION

It is possible that patients with rosacea are more susceptible than normal to climatic stimuli and that the upper dermal changes are a result of these. An increased incidence and degree of elastosis in rosacea patients suggest that loss of integrity of the upper dermal connective tissue may permit vascular dilatation and that this may have an important role in the pathogenesis of the disease (Marks & Harcourt-Webster, 1969).

All the patients described here had similar common male baldness which had been present from 14 to 38 years. It can be supposed that similar conditions developed on the scalp devoid of hair as on the face, thus favouring the occurrence of rosacea from a combination of external and internal factors.

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Pharmacology and Treatment

Evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test

III. HIGH BOILING TAR OIL ACIDS

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Accepted for publication 4 October 1974

SUMMARY

Twelve phenolic fractions of creosote and anthracene oils derived from a high temperature tar were applied in an ointment base to mouse tail skin. After treatment with the higher boiling acids, formerly parakeratotic scale areas underwent granular layer induction and 'basket-weave' keratin was produced. Changes in distribution of acid phosphatase and in horny layer fluorescence were consistent with the conversion to an orthokeratotic state. It is suggested that some of these phenols may be of value in the treatment of chronic psoriasis.

Mouse tail skin has been used as a model of chronic parakeratinization, such as that found in psoriasis (Jarrett & Spearman, 1964; Wrench & Britten, 1975a,b). The latter workers employed this system to evaluate coal tar fractions for anti-psoriatic activity by monitoring for granular layer induction by tar constituents in previously parakeratotic regions. It was found that high boiling tar acids are capable of inducing a granular layer. The acids are located in the fractions known as 'Creosote' and 'Anthracene' oils derived from a high temperature tar. It was decided to locate a possibly therapeutic boiling range of these fractions.

METHODS

Male t. o. mouse tail skin was daily treated with topical applications of the tar preparations as described by Wrench & Britten (1975a). At the end of the tests, the mice were killed, the tail skin removed and subjected to routine histological processing. Seven micron wax sections were cut and stained with haematoxylin and eosin. The epidermal thickness was measured and the skin examined for the presence of a granular layer in the scale regions. Acid phosphatase activity was demonstrated in normal untreated and in treated skin, using the Gomori method described by Pearse (1968). Horny layer fluorescence was observed using the congo red/thioflavine T method of Jarrett & Spearman (1964).

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Tar fractions used

The National Coal Board provided 12 fractions of a mixture of creosote and anthracene oils boiling between 220 and 340°C; these had been 'cut' at each 10°C using a single plate condenser. These oils had been separated from 'Avenue' tar—a high temperature tar. From each of the 12 fractions, the acids were extracted by conventional caustic soda separation as described by Wrench & Britten (1975a).

Formulation

All acids were incorporated into 'Plastibase'*. The Plastibase used comprised 5% polyethylene: 95% liquid paraffin (Robinson, 1955). This vehicle has no effect on the morphology of mouse tail skin (Wrench, 1973).

RESULTS

After seven treatments, it was apparent in the groups listed below that these fractions were having an irritant effect and these mice were killed:

Ten per cent Avenue (anthracene) oil acids (A.O.A.) '220', '240', '270', '310' and '320'.

A tar preparation was considered irritant if the mice displayed 'burrowing' behaviour when approached for treatment, and also after the tar had been applied. The remaining groups did not show this behaviour and so treatment was continued. In groups treated with 10% A.O.A. '220', '240' and '270' the skin was stained very light brown.

After eight treatments, groups treated with 10% A.O.A. '290' and '300' were also killed because of suspected irritant effects.

Ten percent A.O.A. '330'-treated tails began to peel after about six treatments, but there was no sign of distress in the animals. However, it was decided to kill these mice after fourteen treatments as the peeling was by then quite extensive. All other groups at 10% were terminated for comparison.

After sixteen treatments, 5% '330' was causing peeling; all 5% groups were terminated after twenty-one treatments. No irritant effects were observed.

Histology

Histological examination did not reveal any epidermal damage which would account for the apparent irritation in groups treated with 10% A.O.A. '220', '240' and '270'. Epidermal thickness was significantly increased in mice treated with '240' and '270' (Table 1), which may have been manifestations of the supposed irritant reaction. In these two groups, there were a few extensions of the hair follicle granular layer (Jarrett & Spearman, 1964), but otherwise there was no other sign of granular layer induction.

In contrast, groups treated with 10% A.O.A. '310' and '320' showed peeling of the epidermis. In mice treated with 10% '310' there were extensions of the hair follicle granular layer and increases in depth in the naturally occurring interscale granular layers (Spearman, 1964). In 10% '320'-treated mice, peeling was so extensive that epidermal measurements could not be made. No scale granular layers were observed but there were extensions of the hair follicle granular layer in about half the group.

The above observations were paralleled to a lesser degree in mice treated with these fractions at

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* Squibb Ltd.

TABLE I

(%)	Anthracene oil acids	Boiling point range	Days treated	Epidermal thickness (μm)	s.e.	n	t	P
	Control		(21)	27·8	1·21	7	—	—
5	A.O.A. '220'	220–30	21	28·9	1·11	7	0·66	n.s.
10	A.O.A. '220'		7	37·0	1·43	7	4·66	0·001
5	A.O.A. '230'	230–40	21	33·5	1·21	7	3·33	0·01
10	A.O.A. '230'		14	33·5	1·26	7	3·33	0·01
5	A.O.A. '240'	240–50	21	30·5	0·89	7	1·80	n.s.
10	A.O.A. '240'		7	44·8	2·15	7	6·90	0·001
5	A.O.A. '250'	250–60	21	35·2	0·91	7	4·87	0·001
10	A.O.A. '250'		14	32·7	1·75	7	2·32	0·05
5	A.O.A. '260'	260–70	21	37·7	2·45	7	3·64	0·005
10	A.O.A. '260'		14	35·9	2·16	7	3·26	0·01
5	A.O.A. '270'	270–80	21	33·7	0·62	7	4·31	0·005
10	A.O.A. '270'		7	40·2	1·29	7	7·00	0·001
5	A.O.A. '280'	280–90	21	39·5	1·44	7	6·21	0·001
10	A.O.A. '280'		14		Not measurable			
5	A.O.A. '290'	290–300	21	41·9	1·41	7	7·58	0·001
10	A.O.A. '290'		8	50·6	2·50	7	8·23	0·001
5	A.O.A. '300'	300–310	21	50·0	3·12	7	6·67	0·001
10	A.O.A. '300'		8	51·4	2·34	7	8·98	0·001
5	A.O.A. '310'	310–320	21	33·9	0·45	6	4·43	0·001
10	A.O.A. '310'		7	49·1	1·32	5	11·76	0·001
5	A.O.A. '320'	320–330	21	47·1	4·20	6	4·74	0·001
10	A.O.A. '320'		7		Not measurable			
5	A.O.A. '330'	330–340	21		Not measurable			
10	A.O.A. '330'		14		Not measurable			

Initial no. mice/group, 7; weight range, 2·25–25 g.

5%. These animals had been treated for 21 days. Five per cent A.O.A. '220', '240' and '270' caused no change; however there was evidence of peeling in a small area of the sections treated with 5% '310' and '320'. In the latter case, extensions of the hair follicle granular layer were seen and in both cases the epidermal thickness was significantly increased.

Groups treated with 10% A.O.A. '290' and '300' were killed after eight treatments. In both, epidermal thickness was significantly increased and granular layers were induced in parts of the tail. There were also isolated granulated cells just below the horny layer in some scales. Peeling occurred in some areas of the tail in both groups. These fractions at 5% caused increases in epidermal thickness with extensions of the hair follicle granular layer in parts.

The remainder of the groups treated with fractions at 10% were treated for 14 days. Mice treated with 10% '230' and '250' showed significantly increased epidermal thickness, which was also produced by the fractions at 5%, but there was no granular layer formation at either concentration. Epidermal thickness was also increased by A.O.A. '260' at 5% and 10%, and peeling induced by 10% A.O.A. '280' was so extensive that measurements were impossible to make. At 5% there was no peeling, but significant epidermal thickening.

Thickening, peeling and granular layer induction were most obvious in the highest boiling fraction. Measurements were not possible in the case of A.O.A. '330' either at 5% or 10%. At 5% the most obvious effect was peeling; there were a few extensions of the hair follicle granular layer. At 10% there were some very thick and some thin scales, which must have been the result of different rates and degrees of thickening and peeling between individual scales. In some cases the induced granular layers were up to 3 layers thick, and they were continuous over the length of the treated area.

These results are summarized in Table 2.

TABLE 2

Group	% increase in epidermal thickness		Induction of granular cells		Signs of irritation during treatment		Peeling	
	5%	10%	5%	10%	5%	10%	5%	10%
'220'	4	33	–	–	No	Yes	No	No
'230'	21	21	–	–	No	No	No	No
'240'	10	61	–		No	Yes	No	No
'250'	27	18	–		No	No	No	No
'260'	36	29	–		No	No	No	No
'270'	21	45	–	–	No	Yes	No	No
'280'	42	*	–	–	No	No	No	Yes
'290'	51	82	+	+ +	No	Yes	No	Yes
'300'	80	85	+	+ +	No	Yes	No	Yes
'310'	29	77	+	+	No	Yes	Yes	Yes
'320'	69	*	+	+	No	Yes	Yes	Yes
'330'	*	*	+	+ +	No	No	Yes	Yes

– No granular cells induced; + induction of isolated granular cells; + + induction of granular cells forming continuous granular layer; * not measurable.

Acid phosphatase

Tail skin treated with 10% A.O.A. '330' showed a very variable distribution of acid phosphatase compared with normal untreated skin in which acid phosphatase is confined to the parakeratotic horny layer and the hair follicle granular layer. In treated mice, generally it was observed that for haematoxylin and eosin sections displaying induced scale granular layers there were corresponding sections showing acid phosphatase induced in the granular layer region. In some cases, where the horny layers had been retained, it was possible to see that recently produced basket-weave keratin was produced by a layer showing acid phosphatase activity (i.e. the granular layer). The following parakeratotic keratin overlying the thick epidermis was produced without any such layer.

Horny layer fluorescence

Under u.v. radiation after congo red/thioflavine T staining, there were distinct differences in fluorescence of the two types of horny layer present in the tail (Jarrett & Spearman, 1964). In these experiments, the orthokeratin at the neck of the hair follicle fluoresced red and the scale parakeratin fluoresced green.

There was no significant change in mice treated with 5% and 10% A.O.A. '220', '230', '240', '250', '260' and '270'. The response in skin treated with acids in the range 280–340°C was very variable. In some scales there was alternating green and red keratin overlying the scale regions. In the higher boiling acids (310–340°C), the region of the granular layer often fluoresced red, suggesting the beginnings of formation of an orthokeratotic horny layer. In no case did the fluorescence of the horny layer completely change from green to red; the greatest change was in an alternation of green and red scale horny layer.

DISCUSSION

In this experiment, the overall picture seemed at first to be confused by the range of treatment times used. However, some fractions did not induce a granular layer and some were found to be irritant to mouse tail skin. These were acids boiling below 280°C. With fractions boiling above 290°C there was increasing evidence of granular layer induction, but this was accompanied by increased epidermal thickening and peeling. Both of these changes were produced by 10% preparations of the range 280–290°C and thickening was produced by this range at 5%. Granular layer induction was at a maximum in the highest boiling fraction (330–340°C) tested at 10%. However thickening and peeling were also maximized. It was not possible to grade degrees of granular layer induction for these high boiling acids, but it did seem that very active constituents were contained in the highest boiling acids of anthracene and creosote oils.

It was concluded that the acids in the range 280–340°C had a more specific action in inducing granular layers than did the parent tars, oils or whole acids at similar concentrations. These acids were still causing thickening and in some cases extensive peeling of the skin. However, it is thought that these effects highlight even more their specific granular layer inducing abilities and that these adverse side-effects may be controlled by the use of a suitable vehicle.

The results from histochemical demonstrations of tar induced changes reinforced conclusions from granular layer observations, as was predictable from the work of Jarrett & Spearman (1964). These workers pointed out that acid phosphatase often seems to be in abundance when cells are losing their nuclei (as happens during orthokeratinization). Induction of this enzyme in the granular layer region of previously parakeratotic skin occurred after treatment with vitamin A. This activity was similarly induced by the highest boiling fraction of anthracene oil acids (330–340°C), and was also observed in the peripheries of horny layer cells in the newly formed basket-weave keratin. Jarrett & Spearman (1964) have described this distribution and its significance.

In no case did the fluorescence of the horny layer change from green to completely red (as described by Jarrett & Spearman (1964)—after vitamin A and triamcinolone treatment). Strands of red horny layer were often seen interspersed with green orthokeratin, and this too was produced, especially, by the highest boiling acids of creosote and anthracene oils.

These results illustrate the very complex variety of actions on the skin of these acid mixtures, partly due to the large number of compounds still in the fractions, and partly due to the variability in response which can be expected from the action of only one such chemical on the skin. This is evidenced by the variable structure observed within individual mice and the different rates of response of individual scales. Some were found to have full granular layers and also some normal scales still producing scale keratin. This was demonstrated especially by the fluorescence studies. It could be seen that there had been different types of keratinization, in many cases producing a 'sandwich' effect, where orthokeratin alternated with scale keratin. Spearman & Garretts (1966) described such an effect after saline injections into the mouse tail skin, which also caused granular layer induction and mitotic stimulation. They suggested that these effects could be explained by the daily periodicity of treatments, the parakeratosis being partly the result of mitotic stimulation and the orthokeratin being the product of a process which includes the formation of a granular layer. They suggested that the two types of keratin are manifestations of the two effects; i.e. granular layer induction and mitotic stimulation. This also explains the similar effects found in the tar experiments above.

Lewin, Dewitt & Ferrington (1972) similarly related the areas of ortho- and parakeratin in psoriatic fingernails to the presence or the absence of a granular layer.

In experiments where mice were removed daily and killed in an attempt to monitor the effects of the fraction 10% A.O.A. '330' (Wrench, 1973, p. 135), it was noted that maximum granular layer induction preceded maximum development of epidermal thickening. This was also evidenced by the observation that in a rapidly sloughing epidermis—e.g. that produced by 20% preparations of predominantly phenol and cresol mixtures (Wrench & Britten, 1975b)—a granular layer could often be seen in the peeling tissue. Granular layer induction does seem to be one of the primary effects of the tar acids and epidermal thickening is secondary to this.

It is not known whether the epidermal thickening observed was caused by a general increase in cell size and/or by an increase in cell number. Cell hypertrophy was observed in most tar-treated animals, and obviously contributed to the increased epidermal thickness. Mitotic counts need to be carried out to determine just how much mitotic stimulation does occur.

If there had been direct mitotic stimulation, parakeratosis would have been expected, without the formation of a granular layer. It is also possible that peeling caused mitotic stimulation. The suggestion that peeling stimulates mitosis is well known from observations in skin stripping experiments, in which loss of keratinized cells is thought to be the primary stimulus of epidermal cell proliferation (Pinkus, 1952; Allenby, Palmer & Weddell, 1966). Pinkus (1970) also stated that the epidermal thickening seen after skin stripping experiments was due to oedema and hypertrophy of the living cells.

Past work involving granular layer induction both in animals (Bern *et al.*, 1955) and in man (Fry & McMinn, 1968), suggests from the speed with which a granular layer forms (or re-forms) that there must be a direct and primary action of granular layer induction. Bern *et al.* did not obtain granular layers or increases in epidermal thickness with subcutaneous injections of their granular layer inducer —in this case vitamin A. These changes occurred with topical application only. Preliminary experiments with active tar acids suggest that one of their first actions is to induce a granular layer.

It is possible that in the region of the granular layer the acids cause induction but, when they (or their metabolities) penetrate deeper into the epidermis they are potent mitotic stimulators and/or mediators of cell hypertrophy.

With the formation of a more flexible horny layer, the transport of high boiling tar acids across this layer may be increased, with daily increasing amounts of acids reaching the basal layer, and the mitotic stimulatory effects may predominate. However, it has been pointed out that there is not a simple relationship between mitotic rate and the presence or extent of a granular layer (Jarrett & Spearman, 1970). These workers offered an explanation for the seemingly contradictory ability of vitamin A to induce a granular layer and also to stimulate mitosis. It would have been thought that mitotic stimulation would result in parakeratosis. However granular layer induction could have been the result of lysosome rupture in this region, liberating hydrolytic enzymes and thereby initiating orthokeratinization. Such a large enzyme release in the basal layer, amongst very active and therefore highly sensitive cells, would constitute an inflammatory stimulus, one of the many inflammatory reactions being mitotic stimulation.

Such a mechanism is proposed for the high boiling tar acids. This is a most attractive idea, since a common action, i.e. lysosome rupture, is the basis of a granular layer induction and mitotic stimulation. This does not infer any mitotic stimulatory properties of the phenols themselves, which would be a most undesirable side-effect were high boiling phenols to be used in anti-psoriatic therapy.

Similarly skin peeling could be explained by lysosome rupture. Too extensive hydrolysis by the released enzymes could lead to rapid and extensive cell death. The dead cells would be rapidly removed, and the frequent occurrence of several layers of peeling epidermal cells would support this contention.

If the above events do take place, it would perhaps be theoretically possible to prevent mitotic stimulation by employing an inefficient vehicle which only released the acids into the scale horny layer (where lysosome rupture could be initiated), but which did not aid further penetration into the basal layer, where such effects would be injurious.

High boiling tar acids cover a very wide range of substituted polyhydroxyphenols and their isomers (Coal Tar Data Book). They are mostly prepared from low temperature tars since they occur in greater abundance. As early as 1928, Jaffrey found that the most useful coal tar was that containing the greatest amount of acids and that the most important boiling range was 170–300°C, a range which includes both low and high boiling acids. Rothman & Shapiro (1949) stated that the therapeutic fraction of coal tar was the anthracene oil fraction, but they made no special mention of the acids. However, they were referred to by Obermayer & Becker (1935) who suggested that substances such as 8-hydroxyquinoline and catechol should be further investigated. The nature of the phenols in the highest boiling fractions of anthracene oils is unfortunately largely unknown. The next step is to identify specific compounds which cause granular layer induction with the minimum of side-effects. At the present stage granular layer induction cannot be produced without epidermal thickening. However, epidermal thickening relative to granular layer-inducing ability has been reduced in the higher boiling fractions to what it was in the whole acid fractions, and the same applies to skin staining propensities. Skin staining seems to be produced by tar acid and tar oil acid fractions, and although this has not been completely eliminated, it has been reduced relative to possible therapeutic effects. It appears from this work that certain complex phenols in a suitable vehicle could be of great value in restoring chronic parakeratotic plaques in psoriatics to an orthokeratotic state.

ACKNOWLEDGMENTS

This work forms part of that submitted for a Ph.D. thesis by R.W. to Aston University. R.W. would like to acknowledge an S.R.C. postgraduate award and additional financial aid from the National Coal Board.

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British Journal of Dermatology (1975) **93**, 75.

Evaluation of dithranol and a 'synthetic tar' as anti-psoriatic treatments using the mouse tail test

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Accepted for publication 4 October 1974

SUMMARY

Dithranol and 'Psorox'† were evaluated for granular layer induction in parakeratotic regions of mouse tail skin. The lack of this specific activity suggests that they both have different primary actions in the healing psoriatic lesion.

Dithranol (1,8,9-trihydroxyanthracene), has been used as a reasonably safe treatment for psoriasis over the last 50 years (Swanbeck & Liden, 1966), and its use with ultraviolet light is one of the most widely used treatments for psoriasis in Europe (Ippen, 1966). With the increasing awareness of the dangers of prolonged steroid treatments, the use of dithranol in controlling psoriasis has been widely recommended (Shuster & Comaish, 1966; Waddington, 1968; Dahl, 1971; Pegum, 1972). However it should only be applied under medical supervision as it is very harmful to the eyes and can cause severe irritation. Dithranol also stains the skin. It has been found that its anti-psoriatic activity is very much enhanced by incorporation into Lassar's paste (MacLennan & Hellier, 1961). The 'Ingram regimen' involves the use of dithranol in combination with u.v. radiation after a tar bath.

Coal tar treatments have been widely used in the treatment of psoriasis, but their mode of action and active constituents remain obscure (Champion, 1966). The production of individual fractions of tar did not provide a preparation which was widely accepted as being superior therapeutically to crude coal tar (Kerr & Plein, 1953). Some workers have proposed representing the whole tar by a mixture of a few constituents which occur in relatively high proportions in some crude coal tars. Reports of such a tar have been made by Guy, Jacob & Weber (1939), and Butterworth (1950). Similarly, Kinmont & Jowett (1958) tested a synthetic tar which is available as a commercial preparation, Psorox, for the treatment of psoriasis and has the following composition:

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† Fisons Pharmaceuticals Ltd.

Anthracene	4·17%
Naphthalene	41·66%
Phenanthrene	16·66%
Carbazole	10·43%
Picoline	2·08%
Quinoline	2·08%
Pyridine	2·08%
Phenol	4·17%
o-Cresol	4·17%
Toluene	8·33%
Xylene	4·17%
	100·00%

Parakeratotic mouse tail skin has been used as a model of psoriatic keratinization to evaluate anti-psoriatic drugs (Jarrett & Spearman, 1964). These workers showed that anti-psoriatic combinations of vitamin A and triamcinolone caused induction of a granular layer in the scaly regions of mouse tail skin resulting in a change to orthokeratinization. Their model was used to evaluate coal tar fractions for anti-psoriatic activity (Wrench & Britten, 1975a b, c). Certain polyhydroxyphenols were found to produce granular layer induction in this region, and so it was also decided to investigate the effects of dithranol and Psorox for their anti-parakeratotic activity in this model.

MATERIALS AND METHODS

Groups of ten mice were treated once daily with dithranol preparations, Psorox and control vehicles used to formulate dithranol (see below). The ointments were applied all round the proximal half of the mouse tail as described by Wrench & Britten (1975a). The duration of treatment is indicated below. At the end of each experiment, mice were killed and the tail skin removed and then subjected to routine histological processing (Jarrett & Spearman, 1964) and haematoxylin and eosin staining. The skin was examined for the presence of a granular layer in the scale regions and epidermal thickness was measured.

Formulations of dithranol

Since dithranol is used in man at about one quarter of the concentrations used for coal tar (e.g. dithranol paste, B.P.C. 0·1–1·0%; zinc and coal tar paste, B.P.C. at 6%), it was decided to test dithranol at 10%. Forty per cent tar preparations had recently been found to induce tail scale granular layers (Wrench & Britten, 1975a). Dithranol was incorporated into 5% wool fat : 95% yellow soft paraffin, which had been used in the tar experiments, and also into Lassar's Paste.

RESULTS

Psorox

Over 21 days, Psorox did not show any tendency to induce scale granular layers. It caused highly significant thickening of the epidermis (Table 1).

Dithranol

The dithranol ointments did not cause granular layer formation. The epidermis underwent thickening greater than that ever seen after coal tar treatments of mouse tail skin (Wrench & Britten, 1975b, c) (see Table 2).

TABLE 1. Psorox treatment

	Epidermal thickness (μm)	s.e.	*n*	*t*	*P*
Control	35·0	0·90	17	—	—
Psorox	48·0	2·54	10	5·78	0·001

Initial no. mice/group, 10; weight range, 28–32 g; duration, 21 days.

TABLE 2. Dithranol treatment

	Epidermal thickness (μm)	s.e.	*n*	*t*	*P*
Control	23·6	1·20	7	—	—
10% dithranol/Lassar's	63·0	5·52	8	6·56	0·001
10% dithranol/Y.S.P.*	67·3	4·40	9	12·72	0·001
Lassar's paste	26·7	1·06	9	1·92	n.s.

Initial no. mice/group, 10; weight range, 23–27 g; duration, 28 days.
* Y.S.P., mixture of 5% wool fat : 95% yellow soft paraffin.

DISCUSSION

Jarrett & Spearman (1964) have well established both histologically and histochemically the parallels between the parakeratinization in mouse tail scale and in psoriatic epidermis. This model has already demonstrated the ability to induce a granular layer of vitamin A (Jarrett & Spearman, 1964; Spearman & Jarrett, 1974) and of coal tar acids (Wrench & Britten 1975a, b, c). It would be expected therefore that an effective anti-psoriatic drug such as dithranol should similarly induce granular layers in mouse tail skin. Its failure to do so indicates that its primary action in psoriatic skin is not that of granular layer induction. Its action may be secondary to an initial anti-mitotic effect (Swanbeck & Liden, 1966). In increasing epidermal transit time there would be more time for a granular layer to reform (Jarrett & Spearman, 1964). At the high concentrations used in this experiment the mitotic stimulatory effects which dithranol is known to possess (Bock & Burns, 1963) may have predominated over and masked any ability to induce a granular layer.

A lack of specificity is similarly suggested in the case of Psorox, which contains the ingredients described by Kinmont & Jowett (1958). Wrench & Britten (1975a) have shown that the granular layer-inducing constituents of coal tar are phenolic only. About 85% of Kinmont's admixture contains neutral substances. Wrench & Britten (1975a) have shown that neutral substances increase epidermal thickness but fail to induce a granular layer. It was therefore suggested that these compounds play no role in the restoration of orthokeratinization, and that their omission from future coal tar preparations may reduce epidermal thickening effects, particularly unwanted in the psoriatic lesion. The 'synthetic tar' contains about 8% of phenol and o-cresol, both of which have been shown to be of no value in inducing granular layers or producing orthokeratinization in mouse tail skin (Wrench & Britten, 1975b). In the clinical trial of this mixture (Kinmont & Jowett, 1958), there was no histological evaluation, and so a specific effect of inducing granular layers in psoriatic patients is still in question. Young (1970) recently showed this synthetic tar to have no beneficial effect on psoriasis.

During treatment with dithranol, it was noted that it caused staining, which at first was more marked in the Lassar's paste preparation; however the degree of staining in the two groups as a whole was more or less equal at the end of the trials. In all other cases of mouse tail treatment, except the dithranol in Lassar's paste group, all traces of the previous application had disappeared in 24 h. In this case, Lassar's paste could still be seen on the skin when the next treatment was due. Perhaps this persistent adherence on such scaly skin illustrates how dithranol may have a more prolonged effect in psoriasis when in Lassar's paste, thereby being a more potent preparation.

The results show that dithranol and Psorox do not possess the ability directly to induce a granular layer in psoriasis, and hence must act in some other fashion to restore orthokeratinization in psoriatic lesions.

ACKNOWLEDGMENTS

These results form part of Ph.D. thesis (R.Wrench, University of Aston). The work was carried out during the tenure of an S.R.C. Postgraduate Award and with additional financial assistance from the National Coal Board.

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Efficacy of miconazole in the topical treatment of tinea pedis in sportsmen

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Accepted for publication 18 October 1974

SUMMARY

A double-blind trial of 2% miconazole in a cream and in a powder base and of the respective vehicles was done in a group of forty-five young sportsmen regularly training and using the showers in a gymnasium. After 4 weeks treatment, the mycological cure rate using the active preparation was 60% compared to 24% for the placebo ($P<0{\cdot}05$).

Miconazole (1-[2,4,dichloro-β-(2-4-dichlorobenzyloxy)-phenyl] imidazole nitrate) has been shown to have activity *in vitro* against dermatophytes, yeasts, Gram positive bacilli and cocci (Godefroi *et al.*, 1969). At a concentration of 1% in a lotion it has been reported to be highly effective in the treatment of tinea pedis and other dermatophyte infections (Brugmans, van Cutsem & Thienpont, 1970; Botter, 1971). In Britain it is available as a 2% cream and since this is likely to be used in self-treatment of tinea pedis by the general public a clinical trial was arranged to estimate its efficacy in a group of subjects under relatively uncontrolled conditions.

MATERIALS AND METHODS

The subjects selected for the trial were mainly young male university students who regularly train in the gymnasium for soccer, rugby and other sports and who use the associated showers as well as those in sports pavilions. Volunteers were screened mycologically for tinea pedis and those infected were included in the trial. At the start of treatment the presence or absence of peeling, maceration, erythema and fissures was recorded; peeling was also assessed as mild (+) moderate (+ +) or severe (+ + +). Further specimens were taken for laboratory examination from the 4th toe clefts and any other suspicious areas and were processed by culturing twelve inocula on 4% malt extract agar and by microscopic examination in 20% KOH. Since miconazole is only slightly soluble in water, no attempt was made to remove residual cream from skin scrapings taken following treatment. Subjects were requested not to apply the preparation on the day before attending for examination but not all of them obeyed. After the initial investigation, therefore, although culture was also attempted, the emphasis in laboratory diagnosis was placed on direct microscopy and the species of dermatophytes shown in the tables are those isolated at the initial examination.

Two per cent miconazole was supplied as a cream* and as a powder by Janssen Pharmaceutical Ltd. The placebo preparations were the respective vehicles. Both active and placebo preparations were randomly numbered so that neither subject, clinician nor mycologist knew which preparation was being used. Instructions were given to apply the cream to the affected areas by gentle massage each evening, and to apply the powder each morning and after bathing. The treatment period was 4 weeks; after 2 weeks the subjects were examined clinically and after 4 weeks both clinically and mycologically. Those subjects found to be mycologically negative at 4 weeks were re-examined after a further period of 4 weeks during which only the powder was used.

RESULTS

The average age of the subjects was 24 years and the average duration of symptoms of tinea pedis was 3½ years. The use of the gymnasium showers in all subjects averaged 2·5 times per week. Of the fifty-six subjects selected for the trial, forty-five completed the course of treatment; twenty received miconazole and twenty-five the placebo. After 4 weeks treatment, twelve (60%) in the former group were mycologically clear of infection compared to six (24%) in the placebo group (Table 1). After a further 4 weeks, sixteen (89%) of the eighteen mycologically negative subjects were re-examined and eleven (69%) were found to be still free of infection; of these, seven were on miconazole and four on the placebo. Of those which were positive, three were on the active preparation and two on the placebo (Table 2).

TABLE 1. Mycological results after 4 weeks of treatment

Species of fungus	No. of subjects	Active		Placebo		Significance*
		Positive	Negative	Positive	Negative	Active: placebo
Trichophyton rubrum	27	6	7	11	3	n.s.
T. mentagrophytes var. *interdigitale*	10	0	4	4	2	Numbers too small for analysis
Epidermophyton floccosum	4	—	—	4	0	
Hendersonula toruloidea	1	1	0	—	—	
Microscopically positive (no culture)	3	1	1	0	1	
Totals	45	8	12	19	6	$P < 0{\cdot}05$

* Chi squared test; n.s., not significant.

Because the response to topical treatment varies according to the species of dermatophyte, the infections in six subjects which were caused by more than one dermatophyte have not been detailed in the Tables. Instead these have been classified according to the species considered to be most refractory to treatment. *T. rubrum* was considered more difficult to clear than *E. floccosum* which in turn was considered more difficult than *T. interdigitale*. On this basis *T. rubrum* infection occurred in twenty-seven (60%) subjects, *T. interdigitale* in ten (22%) and *E. floccosum* in four (9%). A culture was not obtained from three of the remaining subjects and in one the infection was caused by *Hendersonula toruloidea* (Gentles & Evans, 1970). After the 4-week treatment period a culture was

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* Daktarin

TABLE 2. Mycological results after 8 weeks (subjects negative at 4 weeks)*

Species of fungus	No. of subjects	Active		Placebo	
		Positive	Negative	Positive	Negative
T. rubrum	9	2	4	2	1
T. mentagrophytes var. *interdigitale*	5	1	2	0	2
Microscopically positive (no culture obtained)	2	0	1	0	1
Totals	16†	3	7	2	4

* Numbers too small for statistical analysis.

† Two individuals did not return for follow-up examination.

obtained from only the *H. toruloidea* infection and the positive diagnosis for seven of the eight individuals on the active preparation was therefore made by microscopy. In contrast, cultures were obtained from fourteen of the nineteen subjects with positive microscopy receiving the placebo.

In six (46%) of the thirteen *T. rubrum*, and four (100%) of the *T. interdigitale* infections treated with the active preparations a mycological cure was obtained at 4 weeks, while the results in the placebo group were three (21%) and two (33%) respectively. All the *E. floccosum* infections were in the placebo group and none was cleared. The *H. toruloidea* infection did not respond to the active preparation.

Clinically, after 4 weeks, sixteen (80%) of the subjects receiving miconazole but only ten (40%) of those receiving the placebo were free of infection. When the species of dermatophyte was taken into account, clinical improvement occurred in ten (77%) of the *T. rubrum* infections and in four (100%) of the *T. interdigitale* infections in the miconazole group whereas improvement in the placebo group occurred in six (43%) of the *T. rubrum* infections and three (50%) of the *T. interdigitale* infections. None of the *E. floccosum* infections improved and one deteriorated, possibly due to sensitivity to the base (Table 3).

At the beginning of the trial thirty-three individuals had subjective symptoms. After treatment all fourteen using the active preparation reported absence of symptoms and sixteen (80%) of twenty regarded the treatment as beneficial. In the placebo group nine (47%) of nineteen still had subjective symptoms and only eleven (44%) of twenty-five considered the treatment beneficial.

Only two persons, one using the active preparation and the other the placebo, admitted they did not use the preparations as directed.

DISCUSSION

There are considerable disadvantages in estimating the effect of a topical antifungal agent by self medication. Although 95% of our subjects stated they had used the preparation regularly, the care and diligence with which it was applied would vary greatly with the individual. Moreover, many persons tend to stop or become careless with treatment when discomfort ceases even when asked not to do so, as in this trial. The high proportion (60%) of *T. rubrum* infections and the intense athletic activity of training in the gymnasium during our investigation must have militated against the success of treatment. In addition, the frequent use of the showers (there was an incidence of 37% of tinea pedis in an unselected sample of users) meant there was repeated exposure to infection and our subjects

TABLE 3. Clinical assessment after 4 weeks of treatment

Species of fungus	No. of patients	Active		Placebo		Significance
		No improvement	Improvement	No improvement	Improvement	Active: placebo
T. rubrum	27	3	10	8	6	n.s.
T. mentagrophytes var. *interdigitale*	10	0	4	3	3	Numbers too small for analysis
E. floccosum	4	—	—	4	0	
H. toruloidea	1	1	0	—	—	
Microscopically positive (no culture)	3	0	2	0	1	
Totals	45	4	16	15	10	$P < 0{\cdot}02$

also used showers in sports pavilions where the incidence of tinea pedis among users was likely to be high (Gentles, Evans & Jones, 1974).

The high incidence (13%) of mixed infections which we found is rarely encountered except in groups of individuals frequently using communal bathing places, such as miners using pit-head baths (Gentles & Holmes, 1957), and indicates a high rate of spread of infection. English (1962) believed that most recurrences are due to re-infection rather than relapse. On this basis alone the challenge to miconazole therapy was strong.

Only when symptoms are severe, does the clinical condition of the feet give any indication as to the presence or absence of dermatophyte infection (Holmes & Gentles, 1956). Nevertheless, it is worth noting that, despite the adverse effects of intensive gymnasium activity in rubber soled shoes, the active preparation succeeded in eliminating the subjective symptoms in 100% and objective symptoms in 80% of cases compared to the placebo group in which they persisted in 47% and 60% of cases respectively. It is perhaps also significant that, as also reported by Brugmans *et al.* (1970), positive cultures were not obtained from those in the miconazole group who were shown by microscopy to be still infected after treatment except for *H. toruloidea* which has a sensitivity range quite different from that of dermatophytes. There is the probability, therefore, that they were no longer a source of infection to others using the showers during this period. Whether the continued use of miconazole over a longer period would have resulted in a cure is uncertain.

Our cure rate is less than that reported by Brugmans *et al.* (1970) for miconazole lotion, but their subjects were geriatric in-patients; the lotion was applied by trained medical personnel and was given more frequently during the first week. Furthermore, *T. rubrum* infections, which are extremely difficult to cure, formed only 16% of their cases compared to 60% of ours.

The results of our investigation compare favourably with those reported for other topical antifungal agents tested under similar, or possibly less stringent conditions (Neuhauser, 1964; Katz & Cahn, 1972). The mycological cure rate of 60% using the active preparation is significant compared with the 24% cure rate using the placebo ($P<0{\cdot}05$). Miconazole must be considered as an effective preparation for the treatment of tinea pedis and with the advantage of activity against yeasts and bacteria it is a useful addition to the range of topical antifungals.

ACKNOWLEDGMENTS

We are most grateful to Mr Graham Niven, Director of Physical Education, University of Glasgow for his co-operation; to the Technical Staff of the Mycology Department for their willing assistance with processing of specimens and to Janssen Pharmaceutical Ltd, for supply of miconazole and statistical analyses.

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Granuloma annulare and necrobiosis lipoidica treated by jet injector

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Accepted for publication 25 October 1974

SUMMARY

A Porto-jet injector was employed to treat forty-five cases of granuloma annulare and five cases of necrobiosis lipoidica with triamcinolone acetonide or sterile normal saline. Complete clearance of granuloma annulare lesions was achieved in nearly 70% of those receiving triamcinolone and in 44% with saline. Inadequate penetration of lesions was responsible for a number of failures. The rate of recurrence after treatment was high but retreatment was usually successful. In three cases of necrobiosis lipoidica complete resolution occurred, while in one, partial improvement was obtained. No serious complications of this type of treatment were observed.

Granuloma annulare (G.A.) is a benign condition which frequently presents a therapeutic problem. It runs a variable course before ultimate spontaneous resolution but this course is often very protracted. In a series of 115 cases (Wells & Smith, 1963), only half resolved within 2 years, while the overall duration varied from 3 months to 25 years. In addition, even after spontaneous clearance, 41% of their patients had one or more recurrences, which although usually resolving more quickly than the primary lesion were, none the less, persistent.

Many methods of treatment have been advocated but little information appears to exist about their effectiveness.

Intra-lesional injection has been employed, and in a small series reported by Kern & Schiff (1960) it was suggested that hydrocortisone, saline, sterile water, procaine and xylocaine could be used equally successfully.

Necrobiosis lipoidica (N.B.L.), whether associated with diabetes or not, also undergoes spontaneous healing, but very infrequently (Rollins & Winkelmann, 1960). Topical corticosteroids (Savitt, 1955; Newman & Feldman, 1951) and surgical excision and grafting (Nylen & Skoog, 1958) have been advocated as treatment. Injections of hydrocortisone have also been used with success in four patients reported by Marten & Dulake (1957), using conventional methods.

This study details the results obtained after the treatment of a large series of patients with G.A., using a Porto-jet needleless injector. An attempt has been made to assess both the effect of saline and triamcinolone acetonide and also the frequency of recurrence following clearance induced by treatment. Five cases of N.B.L. are also included.

MATERIALS AND METHODS

Forty-five patients with G.A. and five with N.B.L. were treated. They ranged in age from 8 to 73 years and consisted of 35 females and 15 males. In twenty-seven, the diagnosis was confirmed by biopsy. One patient with G.A. and two with N.B.L. were diabetic, while eight patients with G.A. gave a family history of diabetes. The mean duration of the G.A. was 3 years (range 5 months to 15 years); only six patients were recorded to have had previous spontaneous clearance. Two of the cases of N.B.L. had atypical annular necrobiosis of the face (Wilson Jones, 1971), confirmed by biopsy. One of these had a combination of annular lesions on the back and face, with typical necrobiosis lipoidica on the shins. The other had G.A.-like lesions on the elbows. In none of the cases of N.B.L. had the condition progressed to severe atrophy or ulceration.

Injections of 0·1 ml triamcinolone acetonide (5 mg/ml) or of sterile normal saline were delivered into the G.A. lesions to produce even infiltration. The plaques of N.B.L. were infiltrated around the active margins before the central areas were treated. Great care was taken to avoid injecting areas of marked atrophy. Injections were performed with a Porto-jet needleless injector as described previously (Verbov & Abell, 1970). Initially patients were treated with a series of three injections at 2-weekly intervals, but this was subsequently modified as single injections gave similar results on follow-up. Subsequently injection given at 6- to 8-weekly intervals were performed as indicated by the clinical situation. The number of treatments varied from 1 to 16 (mean 2·4) and the follow up period from 2 months to 2 years (mean 4·5 months). For assessment purposes, results were grouped as follows:

(a) Complete clearance of all treated lesions.
(b) Partial clearance.
(c) No change.

Five cases in which initial partial improvement was followed by rapid relapse were assessed as being unchanged. Twenty-six and twenty-five patients with the nodular form of G.A. received sterile normal saline and triamcinolone acetonide respectively. Eleven patients in each group received both types of therapy for separate lesions. Five patients with diffuse G.A. and the five with N.B.L. were treated with triamcinolone acetonide.

TABLE 1. Injection treatment of granuloma annulare

	Cases	Mean no. treatments	Mean no. injections	Results Complete clearance	Partial clearance	No change	Recurred
Triamcinolone acetonide							
Nodular	25	2·9	32	17 (68%)	6 (24%)	2 (8%)	8 (47%)
Diffuse	5	2·3	54	3	1	1	–
Normal saline							
Nodular	27	1·6	24	12 (44%)	9 (33%)	6 (22%)	5 (42%)
Diffuse	1	1	18	1	–	–	–

RESULTS

Complete clearance of G.A. lesions was achieved in 17 of 25 (68%) treated with triamcinolone (Figs. 1 and 2) and in 12 of 27 (44%) using sterile saline; in both groups the recurrence rate was high, being

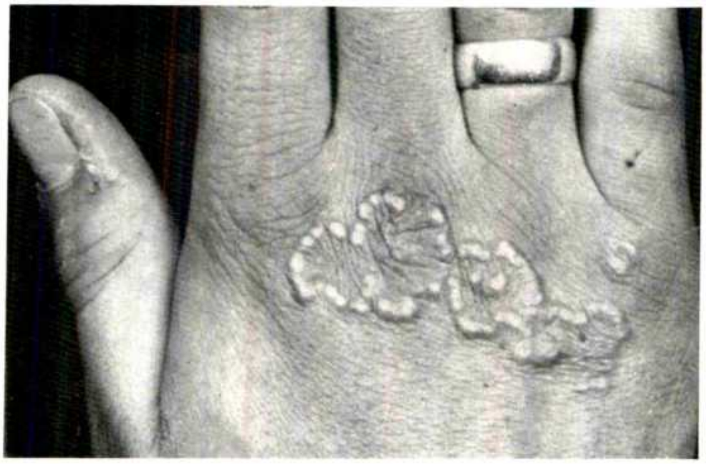

FIGURE 1. Granuloma annulare before treatment.

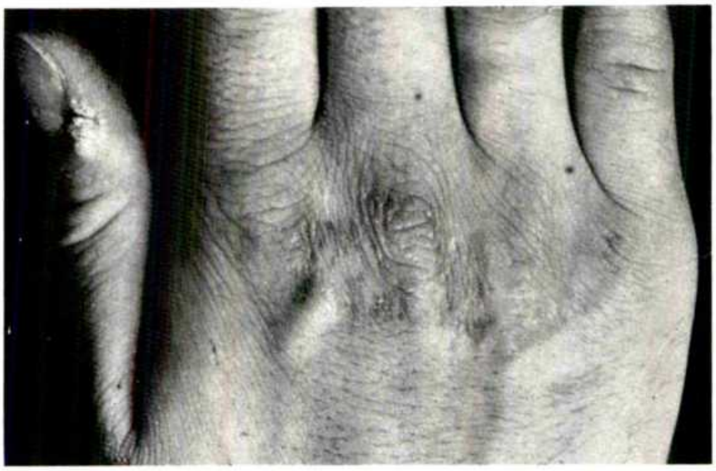

FIGURE 2. Granuloma annulare 5 months after treatment (triamcinolone acetonide).

8 and 5 respectively. Resolution usually took 2–4 weeks after successful injection and recurrences appeared within 3-7 months of the time of initial treatment. Thirteen of these patients with recurrent lesions were retreated, only one of whom failed to respond.

Certain patterns of response appeared during the follow-up. Over half the patients responded by rapid and complete clearance with a small number of injections (1–3). In a small group improvement occurred in the sites injected coincidental with the development of new lesions elsewhere. This led to prolonged periods of treatment. Among the eleven cases treated by both methods in separate episodes of G.A. the therapeutic response to triamcinolone and saline appeared the same in four, but the rest cleared more readily with triamcinolone.

The diffuse G.A. cases appeared to respond in a similar way to the nodular forms.

Three of the five cases of N.B.L. showed gradual resolution after a small number of treatments.

Subsequent observation over 1–12 months (mean 8) failed to show signs of recurrence. Slight hyperpigmentation persisted in the areas of treatment, but progressively faded. A patient with classical N.B.L. of the shins had repeated injections over a 2-year period, with gradual but partial improvement. The patient unchanged by treatment had atypical necrobiosis of the face alone. This spontaneously cleared during a pregnancy, only to relapse during the puerperium.

Complications

Transient atrophy at the injection sites of triamcinolone was an almost constant finding in resolving G.A. lesions. However, even saline treated lesions often showed some erythema on clearing.

In one case of N.B.L. multiple tiny ulcers appeared at the injection sites. These healed spontaneously less than 3 months later and subsequent injections were given cautiously and without further complications.

In certain situations great difficulty occurred in penetrating into G.A. lesions. Nodules of the fingers, especially those set around the interphalangeal joints, were often inadequately infiltrated. Repeated attempts at injecting these sites were sometimes successful, but a proportion of failures was due to this problem.

Some slight discomfort is produced by this injector but it was never sufficient to prevent continued treatment.

DISCUSSION

The lack of substantial information on the efficacy of treatment in G.A. makes it impossible usefully to compare these results with previous reports. However, Kern & Schiff reported success in 3 of 4 cases treated with a number of compounds, while Wells & Smith commented that the rate of resolution did not seem to be affected by various treatment methods, or by biopsy. These treatments included vitamin E (27 cases), elastoplast occlusion (19), CO$_2$ snow (13), X-ray (8) and intralesional hydrocortisone (4).

Our results suggest that nearly 70% of patients with G.A. injected with triamcinolone can expect to gain complete resolution promptly. Saline injections were noticeably less effective but frequently produced an initially better cosmetic effect due to the lack of atrophy. Treatment with saline at first is probably well worthwhile before using triamcinolone.

The high recurrence rate in each treatment group corresponds closely with the figure of 41% for natural recurrence described by Wells & Smith (1963). Although these figures are high, the method does allow for rapid retreatment.

The use of triamcinolone injections in N.B.L. certainly appears to be worthy of consideration, particularly if the condition can be treated before the onset of severe atrophy and ulceration. Whether injection of such advanced cases is justifiable seems doubtful. Our results seem to compare well with those obtained by Marten & Dulake, and, with care, ulceration from injection can be prevented.

ACKNOWLEDGMENTS

We wish to record our thanks to the many physicians at St John's Hospital who referred their patients for treatment.

We are also indebted to Mr Waller for supply of sterile fluids for injection and to the Department of Medical Illustration for the illustrations.

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British Journal of Dermatology (1975) **93**, 91.

Case Reports

Pseudoxanthomatous mastocytosis

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Accepted for publication 5 October 1974

SUMMARY

A case of xanthelasmoidea (pseudoxanthomatous mastocytosis) occurring in a 50-year-old Iranian man is described. The patient had a large upper gastrointestinal haemorrhage.

In 1875 Tilbury Fox described three cases of a new dermatosis, which he called xanthelasmoidea, consisting of a profusion of small cutaneous nodules with a yellowish colour. The eruption was later recognized as an uncommon variant of urticaria pigmentosa. The few subsequent reports have been of children or young adults. We have had the opportunity to study a case of long-standing and to observe one of its major complications.

CASE REPORT

An Iranian male aged 50 years with multiple cutaneous nodules and loss of the outer half of his eyebrows was referred to the University Hospital with the suspicion of leprosy. The nodules had been present since birth and were associated with intermittent episodes of itching, but no blistering. No members of his family had any cutaneous disorders. He had also suffered from epigastric pain for 1 month, and melaena for 3 days. He had not previously had gastro-intestinal symptoms, and he had not suffered from headaches. Before he could be referred to the specialist clinic for leprosy he had a massive upper gastro-intestinal haemorrhage and was admitted as an emergency.

On examination his cutaneous surface was covered with a profusion of yellow or cream coloured nodules varying in size from 1 mm to 2 cm in diameter (Figs. 1 and 2). They had a soft doughy consistency and could be indented by the palpating finger in the same way as localized anetoderma. The loss of eyebrows was due to breakage of the hairs from rubbing his face. On the lower limbs many of the nodules were perifollicular in distribution. His spleen was enlarged 12 cm below the left costal margin, but hepatomegaly and lymphadenopathy were not present. At this stage a tentative diagnosis of pseudoxanthoma elasticum was made, but angioid streaks were not seen in his retina. Examination of all other systems was normal. His peripheral blood reflected the recent blood loss. Liver function tests were all normal. Because of excessive bleeding, upper gastrointestinal barium studies were tech-

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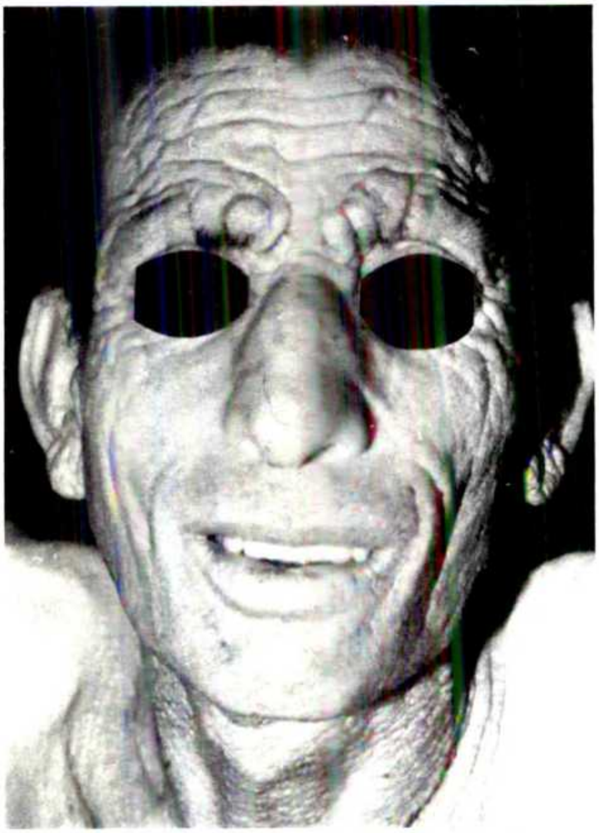

FIGURE 1. Profuse nodular thickening of the skin especially marked around the neck and on the ears. The eyebrows are lost.

nically unsatisfactory and the site of bleeding was not determined. He declined more extensive investigations.

A biopsy of one of the nodules showed a very dense round-cell infiltrate in the dermis (Figs. 3 and 4). Special stains showed this to be composed of mast cells, and a diagnosis of urticaria pigmentosa xanthelasmoidea type was made. The cutaneous lesions became red on friction but did not urticate.

COMMENT

Knowledge of the multinodular non-pigmented variant of mastocytosis with lesions resembling xanthomata has developed over the last 100 years. In 1869 Nettleship described the first case recognized as urticaria pigmentosa in a child from the Blackfriar's Skin Hospital. Tilbury Fox (1875) presented the first multinodular case which he called xanthelasmoidea. Thin (1877) showed that the skin in ordinary urticaria pigmentosa had a dense cellular infiltrate, but he wrongly concluded that it was an early manifestation of lupus (vulgaris).

The term urticaria pigmentosa was proposed by Sangster (1878); T. Colcot Fox (1883) gave follow-up details of Tilbury Fox's case and demonstrated that the histology was that of an urticaria. The cellular infiltrate was shown by Unna (1887) to be composed of mast cells.

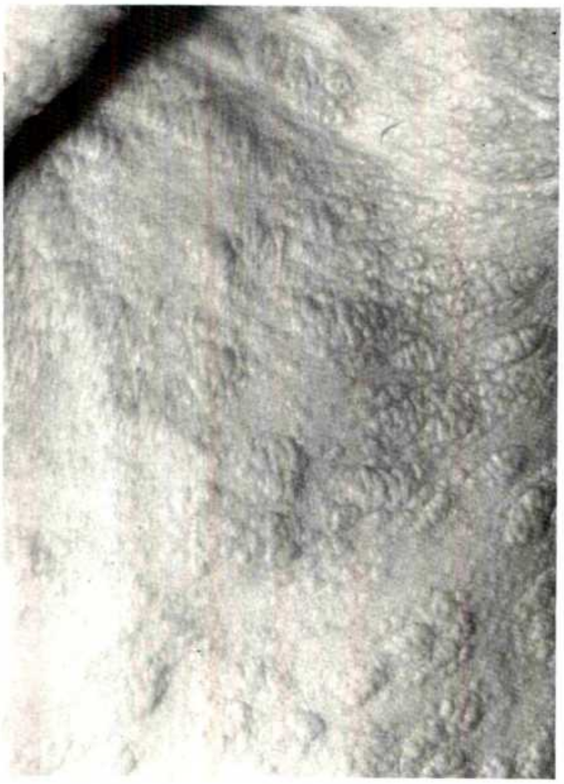

FIGURE 2. Close-up of left axilla. Numerous soft anetoderma-like nodules, formed by the coalescence of smaller perifollicular elements.

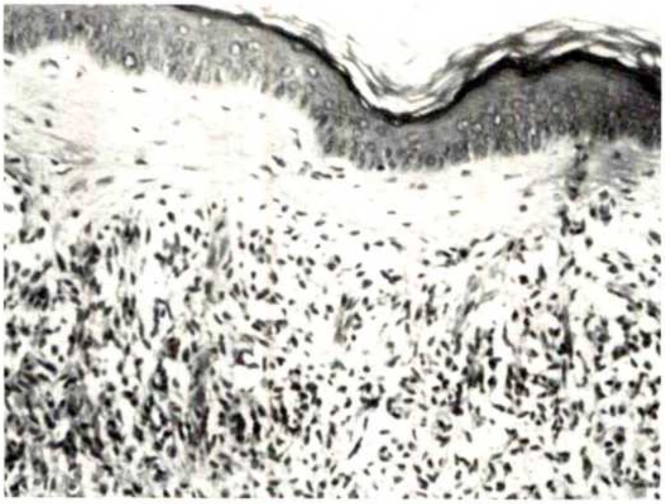

FIGURE 3. Skin shows diffuse mast cell infiltration in the deep dermis. The upper dermis shows no cellular infiltration. (H & E × 150.)

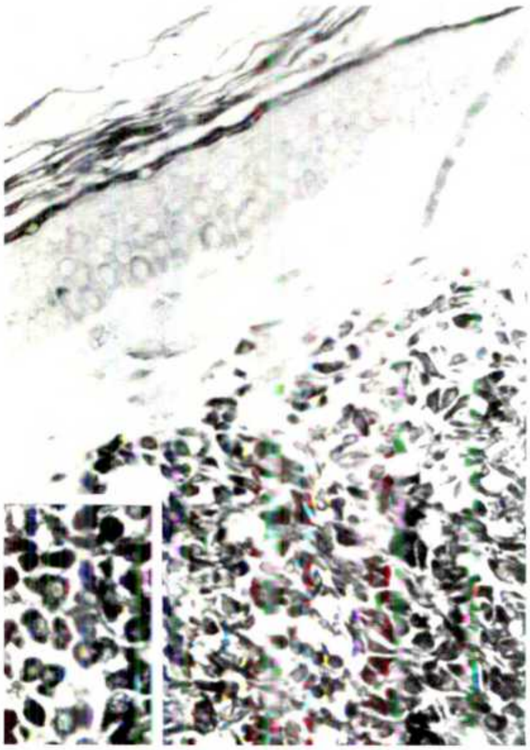

FIGURE 4. Metachromatic stain demonstrates dark irregular cytoplasm of the mast cells. The nuclei are pale. (Toluidine blue × 350.) Inset: high magnification of the mast cells showing the dark metachromatic granules in the cytoplasm. The nuclei are not stained. (Toluidine blue × 720.)

Up to 1923, eleven cases of the xanthomatous type had been reported in the literature representing 8% of the cases of urticaria pigmentosa (Finnerud, 1923). Gougerot, Burnier & Eliascheff (1931) outlined the clinical features of the xanthomatous type and Sézary, Lefèvre & Chauvillon (1935) stressed that pigmentation was not a necessary feature; he also suggested the term mastocytoma for this group of diseases. Degos & Vissian (1949) gave a clear description of the multinodular xanthomatous type, stressing the complete absence of pigmentation; Degos (1974) added to the description and provided illustrations.

Demis (1963) mentioned the frequency of peptic ulceration in urticaria pigmentosa. Haematemesis is a complication listed by Parker & Odland (1971).

Tilbury Fox's case 'presented a curious appearance and was literally as spotted as a leopard, only in place of discolourations were these buff-coloured nodules or nodose infiltrations studding the white normal skin, interspersed with the dull reddish blotches. . . . In some cases the nodules have shrivelled more or less, leaving a pale-fawn coloured slightly elevated patch. . . . On close inspection the patches are seen to be made up of a congeries of indistinctly marked small projections which seem to be seated at the hair follicles. . . .'

The present case very closely resembled this first description, but differed from it in failing to resolve after a few years. Our case is unusual in still having numerous lesions after 50 years and eventually suffering from a massive upper gastro-intestinal haemorrhage which may or may not have been related to his urticaria pigmentosa.

The similarity of the lesions both to xanthomas and to pseudoxanthoma elasticum is striking. 'Xanthelasmoidea' only partly conveys our knowledge of this disease, so perhaps a better descriptive term for this variety of mastocytosis would be pseudoxanthomatous mastocytosis.

ACKNOWLEDGMENTS

We would like to thank our Iranian colleagues for permission to study this patient and Dr S.C.Gold for advice on the preparation of the manuscript.

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British Journal of Dermatology (1975) **93**, 97.

Urticaria and hyperthyroidism

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Accepted for publication 14 October 1974

SUMMARY

A thyrotoxic patient presented with urticaria and this appeared to respond to beta-blockade with practolol. Symptoms did not recur when radioactive iodine treatment took effect in spite of stopping the beta-blocker. Possible mechanisms are discussed and it is stressed that more emphasis should be placed on this association.

Speaking at the VI International Dermatological Congress in New York, 67 years ago, Ravitch (1907) discussed the thyroid as a factor in chronic urticaria. He emphasized that while patients with atrophic thyroid glands did best with thyroid extract, when 'abnormal functions of the thyroid and urticaria were due to thyroid oversecretion, the Roentgen treatment induced unmistakable benefit'. This was probably the first report of urticaria associated with hyperthyroidism and responding to treatment of the thyroid dysfunction.

Subsequently Weiner (1947) mentioned urticaria as an occasional manifestation of hyperthyroidism, but the association somehow remained dormant until interest was renewed following a paper by Isaacs & Ertel (1971). These workers described two patients with generalized pruritus and four with urticaria who also had hyperthyroidism. The skin manifestations responded only to treatment of the thyroid dysfunction. Most endocrinological and dermatological text-books do not mention this association, or merely gloss over it. In 'Dermatology in General Medicine' (Freinkel & Freinkel, 1971), the association is dismissed as probably fortuitous, and it rates no mention in reviews of unusual clinical manifestations of hyperthyroidism (Chapman & Maloof, 1956; Locke, 1967).

CASE REPORT

A 40-year-old Caucasian female was referred to the out-patient clinic because of severe generalized urticaria of 8 weeks duration, and this was completely unresponsive to massive doses of antihistamines. For 12 months there had been increasing irritability and loss of a few pounds in weight. Her mother was thyrotoxic. On examination there was generalized urticaria but mucous membranes were unaffected.

She was clinically hyperthyroid. Her pulse was 110 per minute and there was fine tremor of the outstretched hands. The thyroid was diffusely enlarged with an overlying systolic bruit. There was bilateral lid-lag with fine nystagmus on lateral gaze. Investigations confirmed thyrotoxicosis:

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Protein-bound iodine 9·0 $\mu g/100$ ml (normal 4–8); T3 (serum ^{125}I—triiodothyronine binding capacity) 83% (normal 92–117) i.e. in thyrotoxic range; ^{131}I uptake at 2 h 45%; Thyroid antibodies, absent.

MANAGEMENT

Radioactive iodine therapy was arranged for 10 days later but pending that the patient was started on practolol 100 mg twice daily. Within 3 days a dramatic improvement occurred, and the urticaria had disappeared completely 3 weeks later. Practolol was continued for 2 months after radioactive iodine administration. At this time the patient was both clinically and biochemically euthyroid, and there has since been no recurrence of urticaria.

DISCUSSION

As mentioned previously, the association of urticaria with thyrotoxicosis is uncommon. Rothfield (1968) found two patients with urticaria from a series of 108 thyrotoxic patients and apparently the severity of the skin manifestations varied with the control of the hyperthyroidism. Our patient, like those described by Isaacs & Ertel (1971), was a female; again, although in retrospect symptoms of hyperthyroidism had been present for many months, it was the dermatological problem that drove the patient to seek urgent medical advice.

Like ours, none of these patients had endocrine ophthalmopathy or pretibial myxoedema.

The cause of urticaria (and also of pruritus) in hyperthyroidism is unknown. The wealing was not effort or heat induced and this is against a cholinergic mechanism (Thompson, 1968).

Other possibilities were well reviewed by Isaacs & Ertel (1971) and also lately by Barnes, Sarkany & Calnan (1974) (a review of pruritus in thyrotoxicosis). Authors have usually considered both urticaria and pruritus together but there has been no evidence to show that the mechanism is identical.

A direct long acting thyroid stimulator effect seems unlikely since high *LATS levels, when present, remain elevated even after thyroid ablation (Volpe *et al.*, 1969), and urticaria has not been found in patients with the highest LATS levels, i.e. those with pretibial myxoedema (Kriss, Pleshakov & Chien, 1964).

Thyroid antibody titres are highest in auto-immune thyroiditis (Rose & Witebsky 1968) in which urticaria has not been reported.

In the absence of any benefit from antihistamines a further possibility considered by Isaacs & Ertel was increased kinin activation mediated perhaps by raised catecholamine levels in tissue or blood.

It was interesting on this account that beta-receptor blockade has been found to relieve many of the clinical manifestations of thyrotoxicosis (Riddle & Schwartz, 1970), including pruritus (Eliakim & Rachmilewitz, 1959) without affecting biochemical indices of thyrotoxicosis.

Our own patient responded immediately to beta-blockade and it would seem that sometimes at least, the same mechanism could be operative in both pruritus and urticaria associated with uncontrolled hyperthyroidism. This has, to our knowledge, not been previously reported.

It seems evident that general physicians and dermatologists alike should be made more aware of this presentation of urticaria as a manifestation of thyrotoxicosis, first reported nearly 70 years ago but surprisingly forgotten thereafter.

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* LATS—long acting thyroid stimulator.

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British Journal of Dermatology (1975) **93**, 101.

Recurrent necrosis of the skin and the mouth

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Accepted for publication 26 October 1974

SUMMARY

A patient suffering from an unusual syndrome, in which the following triad was present, is described:

(1) Recurrent necrosis of the skin and mouth; and necrosis following intradermal injection of saline.
(2) Disappearance of the necrotic episodes following the administration of prednisone.
(3) Mental retardation.

This syndrome does not appear to have been previously reported.

CASE REPORT

A 52-year-old, married, white female presented with an ulcer on her right leg. For 8 years she had had recurrent necrosis and ulceration of the mouth and skin (left and right thighs, left leg, right arm, right hand and abdomen). This necessitated a total of twenty hospitalizations with an average stay of approximately 2 weeks. Recurrent necrosis of the skin of the right hand, including the exposed tendons and bones, and resulting infection, had necessitated amputation of the right index, middle and ring fingers. No similar condition was discovered in her family (including eight brothers, five sisters and four children).

On examination, she had an ulcer 6 cm in diameter over the anterior aspect of the right leg, with a necrotic edge and base (Fig. 1). In addition, multiple areas of necrosis and ulceration were found on the tongue (Fig. 2). These were covered with fibrinous exudate of a dirty grayish colour and surrounded with bright red areas of inflammation. The tongue was extremely painful making eating and speaking difficult. The stump of the third metcarpal bone was covered with an ulcerated skin. Scars of previous ulcers were found around the mouth, on the left leg, abdomen, both thighs, and right arm. Her I.Q. was 53. She was also schizophrenic and was on amitriptyline, chlorpromazine and diazepam.

Investigations

(a) *Normal or negative results.* Hb, WBC, platelets; prothrombin time, clotting time, bleeding time, clot retraction, partial thromboplastin time; blood culture; BSP retention; glucose tolerance test; blood urea, serum proteins, creatinine, alkaline phosphatase, bilirubin, cholesterol; EEG; ECG; X-ray skull, chest and abdomen; latex fixation tests, W.R. and V.D.R.L., ANF and auto-antibodies;

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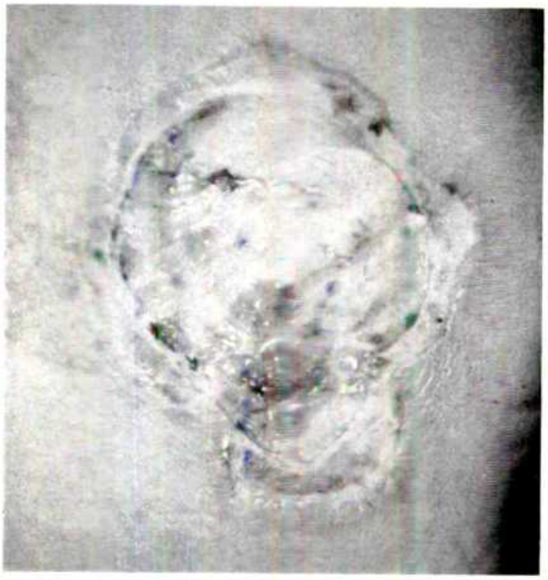

FIGURE 1. Recent ulcer on the right leg.

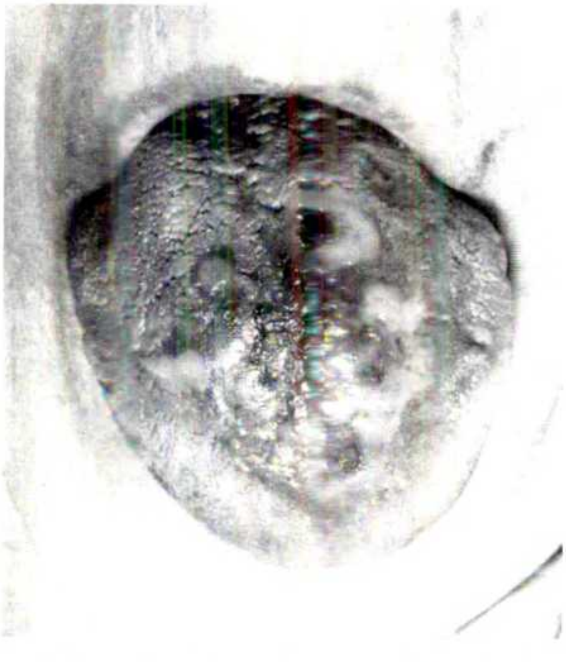

FIGURE 2. Necrosis and ulceration of the tongue.

complete urinalysis (specific gravity, pH, albumin, sugar, coproporphyrin, amylase, bacteria and cells); serum protein electrophoresis and immunoelectrophoresis (gamma A was within upper normal range; no myeloma type of proteins, no cryoglobulin, no monoclonal bands, and no antibodies were detected).

(b) *Abnormal investigations*. ESR varied between 22 and 33 mm/h. Diphtheroids were grown from the necrotic areas. Biopsy of the lesions showed necrosis of the skin, in addition to fat necrosis of the subcutaneous tissue. There was no evidence of vasculitis or any other vessel changes and no cellular infiltrate.

Treatment and Progress

The ulcer on the right leg was debrided and skin grafted. The stump of the third metacarpal bone was shortened and a linear repair was performed. Seroma recurred three times underneath the graft, necessitating evacuation each time. The skin graft then took almost completely. A few days later, a skin test was performed. It consisted of pricking the skin of the arm with a sterile needle at one spot, and intradermal injection of 0·1 ml of saline at another spot. Twenty-four hours later, there was some bruising at the site of the skin prick and actual necrosis at the site of the saline injection (Fig. 3).

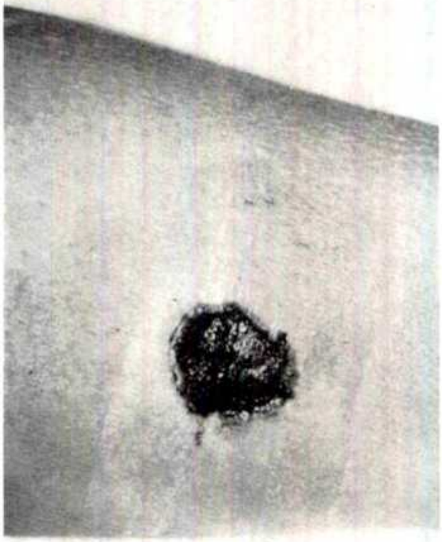

FIGURE 3. Skin necrosis following intradermal injection of saline.

Two weeks later, the tongue lesion had disappeared and the necrotic area on the arm was much smaller. One month later, the lesions on the tongue, lip and arm recurred. The patient was then put on prednisone 20 mg daily. This was tapered and discontinued over a period of 1 month. At that time, the necrosis had disappeared at all sites.

DISCUSSION

Due to the mental retardation of the patient, the lesions were misdiagnosed as self-inflicted; however, a protective plaster cast applied over the necrotic areas failed to change the course or prevent the recurrence of necrosis. In addition, the skin test had excluded the diagnosis of factitial disease. Discontinuation of the psychotropic drugs failed to alter the progress or prevent the recurrence of necrosis. The differential diagnosis included:—

(A) *Pyoderma gangrenosum*

(1) *Skin lesions.* In pyoderma gangrenosum a papulovesicle appears which becomes necrotic and produces painful, undermined ulcers (Brunsting, Goeckerman & O'Leary, 1930) with marked leukocytoclastic vasculitis (Thompson *et al.*, 1973). The skin ulcers in recurrent necrosis of the skin and mouth were not painful, not undermined, nor associated with vasculitis. They were less florid and more necrotic.

(2) *Associated conditions.* Pyoderma gangrenosum may be associated with one of the following conditions:

Ulcerative colitis in 50–60% of cases (Brunsting *et al.*, 1930); local enteritis (Stathers, Abbott & McGuinness, 1967); rheumatoid arthritis (Philpott, Goltz & Park, 1966); viral infection (Van der Meiren, Achter & Kestelyn, 1963); focal sepsis (Hofstad, 1959); diabetes (Philpott *et al.*, 1966); hypogammaglobulinaemia (Karltorp, 1963); monoclonal gammopathies preceding multiple myeloma (Van der Sluis, 1966; Jablonska, Stachow & Dabrowska, 1967; Thivolet & Beyvin, 1967; Cream, 1971); alteration in humoral or cellular immunity (Long & Uesu, 1964; Delescluse, Bast & Achten, 1972).

We are unable to detect any of these conditions associated with recurrent necrosis of the skin and mouth.

(B) *Behçet's syndrome*

(1) *Skin lesions.* In Behçet's syndrome there may be pyoderma in the form of pustules, impetigo, superficial and deep folliculitis, furuncles, cellulitis or simple ulceration (Berlin, 1960); histology usually shows a vasculitis (Domonkos 1971). In recurrent necrosis of the skin and mouth, the necrosis was much more extensive with no vasculitis.

(2) *Associated conditions.* The diagnosis of Behçet's syndrome is usually considered to require the presence of at least two of the following triad of symptoms: recurrent oral ulceration, recurrent genital ulceration and ocular inflammation (Francis, 1970). Ocular symptoms are the most important and the most disabling of the three major components and are present in 70–85% of patients with the disease (Haim, 1968). Intracutaneous injection of physiological saline often produces a papule or pustule (Berlin, 1960; Katzenellenbogen, 1946). Sometimes there is an eruption identical with that of erythema nodosum, with intense accumulation of polymorphs and increased vascular permeability (Yazaki, 1970). In addition, the syndrome may be associated with a cryoparaprotein (Fiddes & Penny, 1971).

In recurrent necrosis of the skin and mouth, genital and ocular inflammation were absent. Skin testing produced more extensive necrosis with less local inflammation. No cryoparaprotein was detected.

(C) *Purpura fulminans*

(1) *Skin lesions.* In purpura fulminans, the lesions begin as bruises which become darker blue, then black and necrotic (Elsahy, 1974).

Sudden and complete necrosis occurred in recurrent skin and mouth necrosis.

(2) *Associated condition.* Purpura fulminans is usually fatal, while recurrent necrosis of the skin and mouth was not fatal in the case reported.

Experience with this patient indicated that corticosteroids had a beneficial effect in both controlling and preventing the spread of skin necrosis.

ACKNOWLEDGMENTS

I wish to thank Drs L.Gazley, R.Banerjee, D.S.Horoupian and L.S.McMorris for their assistance.

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British Journal of Dermatology (1975) **93**, 107.

Methotrexate pneumonitis in a psoriatic

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Accepted for publication 30 October 1974

SUMMARY

A life-threatening respiratory disease, characterized by fever, cough, dyspnoea, cyanosis and widespread bilateral pulmonary infiltrates, developed in a 40-year-old patient receiving intermittent methotrexate therapy for psoriasis. Blood eosinophilia accompanied the illness. A prominent feature in the clinical picture was a discrepancy between auscultatory findings and the pronounced changes in the chest X-ray. Systemic corticosteroids produced rapid improvement. It is suggested that the pulmonary syndrome represents a hypersensitivity reaction.

Methotrexate has been proved of great value in the treatment of severe psoriasis during two decades. It is known to be toxic to several organ systems and different side effects have been reported, e.g. leukopenia, thrombocytopenia, gastro-intestinal bleeding, stomatitis, liver damage and chromosome abnormalities (Nyfors & Brodthagen, 1969; Baker, 1970; Zachariae, 1971).

This report describes a patient in whom a life-threatening pneumonitis presumably was caused by methotrexate taken for psoriasis.

CASE REPORT

A 40-year-old female teacher was admitted to the dermatology department because of fever and dyspnoea occurring during methotrexate treatment for severe psoriasis.

There was no history of previous pulmonary disease and no known exposure to birds, but the patient had a somewhat impaired kidney function due to chronic pyelonephritis. She had not received other drugs than methotrexate; nitrofurantoin had not been given for over a year.

Her psoriasis began at the age of 7 years and during the last 6 years she had widespread and extensive lesions and was repeatedly hospitalized. From January 1973 she was treated with methotrexate, with small divided oral doses (2·5 mg twice with 12 hours interval, once weekly). This dosage was chosen, due to the impaired kidney function; her serum creatinine was 1·9 mg/100 ml and creatinine clearance was 28 ml/min. After partial remission the dosage was reduced to 2·5 mg weekly, and interrupted with intervals, partly following remissions and partly due to side-effects of nausea and stomatitis.

In May 1974 she was readmitted to hospital with general malaise, fever, cough and shortness of breath. Her leukocyte count had dropped to $1200/mm^3$ and platelets to $40{,}000/mm^3$ 2 days before

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admission. On admission auscultation was normal, the blood pressure 130/80 mm Hg, serum creatinine 1·8 mg/100 ml. Several blood cultures were negative. In spite of normal auscultation a chest X-ray showed severe infiltration on both sides (Fig. 1). The leukocyte count had risen to 3100/mm^3 with 22% eosinophils; the platelet count was 195,000/mm^3.

Treatment was initiated with ampicillin and prednisone. In spite of treatment the clinical condition deteriorated and the patient had to be transferred to the intensive care unit, where she was tracheotomized and placed in a respirator. The infiltration of the lung diminished considerably in a few days and disappeared little by little. The general condition improved with the lung function. Respirator treatment was discontinued after 9 days, systemic steroids after 12 days and ampicillin after 16 days.

During treatment, further cultures were taken from blood, trachea, throat and urine, all showing no growth. Serological tests for influenza, adenovirus and mycoplasma pneumoniae were negative. Also tests for cold agglutinins, antinuclear factor and LE cells were negative. Complement C-3 and C-4 were normal on the eleventh day of treatment (0·49 g/l and 59 units). Twenty-one days after treatment both were still normal, but somewhat higher (0·56 g/l and 82 units; normal range 0·37–0·79 g/l and 40–135 units). Serum bilirubin, alkaline phosphatase and GP transaminase showed signs of transitory liver damage, but all returned to normal.

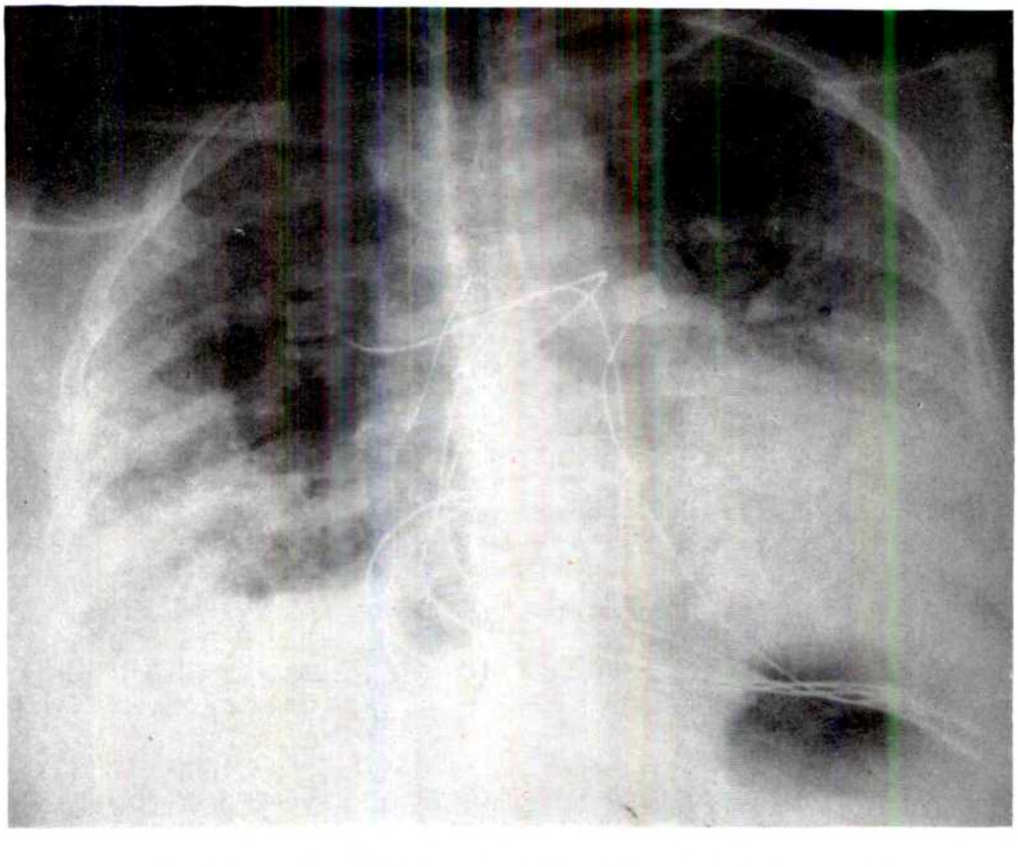

FIGURE 1. Chest X-ray showing pneumonitis with extensive infiltrations on both sides.

DISCUSSION

The case reported in this paper is similar to other cases of pulmonary disease described in patients treated intermittently with methotrexate for leukaemia (Acute Leukemia Group B, 1969; Clarysse *et al.*, 1969; Robertson, 1970; Schwartz & Kajani, 1969). Our patient showed extensive infiltration on chest X-ray, she was desperately ill, dyspnoeic, and deeply cyanotic with insufficient respiration when tracheotomized and placed in a respirator. None of the investigations performed indicated infection, collagen disease or occupational lung disease. However, a drug reaction could explain the clinical picture. She had been on methotrexate for 16 months, receiving a total dose of 215 mg. A dramatic improvement followed when methotrexate was withheld and steroid treatment initiated.

Most of the previous cases with pneumonitis associated with methotrexate occurred in patients with acute lymphoblastic leukaemia in remission (Acute Leukemia Group B, 1969; Clarysse *et al.*, 1969; Robertson, 1970; Schwartz & Kajani, 1969). Similar findings have been reported by Goldmann & Moschella (1971) in two patients treated with methotrexate for pemphigus vulgaris and mycosis fungoides respectively, and by Filip *et al.* (1971) in a psoriatic after 5 years of weekly methotrexate therapy. This case was associated with liver disease and hilar and paratracheal adenopathy.

It has been a common finding among the cases reported by Goldmann & Moschella (1971), Robertson (1971) and Schwartz & Kajani (1969) that auscultation was normal or almost normal in spite of a very pathological chest X-ray. Eosinophilia was a prominent feature in seven of ten patients (Clarysse *et al.*, 1969; Goldmann & Moschella, 1971; Schwartz & Kajani, 1969). Most patients were severely ill and three died (Acute Leukemia Group B, 1969). Without steroids the symptoms lasted between 10 and 40 days regardless of whether or not methotrexate was continued (Clarysse *et al.*, 1969). However, in one of their patients, who received a second single dose of methotrexate, a temporary exacerbation of the respiratory syndrome was seen (Goldmann & Moschella, 1971). Systemic steroids seem to be the treatment of choice (Robertson, 1970; Schwartz & Kajani, 1969). Pasquinucci, Ferrara & Castellari (1971) mentioned that intravenous administration of daunorubicin hastened recovery.

The nature of the pulmonary disease has not been established. Lung biopsy in one patient showed a granulomatous reaction with mild perivasculitis (Clarysse *et al.*, 1969) and resembled lesions described in cases of pigeon breeders' disease (Fink *et al.*, 1968). This together with the prompt improvement after steroid treatment suggests a hypersensitivity reaction.

It is important to recognize this life-threatening illness, so that prompt treatment may be initiated. Helpful diagnostic features are eosinophilia in the blood and a discrepancy between the pronounced changes in the X-ray and the few abnormal signs found by auscultation.

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British Journal of Dermatology (1975) **93**, 111.

Comment

THE PSORIASIS SUFFERER IN THE COMMUNITY

Lucky the patient who receives more than instant diagnosis, a brief explanation and an ointment for psoriasis. He may be sent to a treatment unit where various routines and perhaps time-consuming rituals are followed. With so few dermatologists employed in the NHS it may be difficult to provide a better service and indeed to find the time and opportunity to discover what the consumer really wants.

Based on letters from more than ten thousand patients, the Psoriasis Association of Great Britain feels some anxiety that consumers are not getting what they need and that dermatology is not 'pressing the true cure' (Francis Bacon, 1597), by being far too complacent about its present management of the patient, perhaps too selfcongratulatory of its advancement in the understanding of the disease and through paying more attention to the rash and its transient and occasional removal, than to the problems of the patient in the community in relationship to that rash.

One third of all patients have spontaneous remissions (Farber, Bright & Nall, 1968) and some treatments may even prevent such remissions (Ryan & Baker, 1971). It is possible that attention to the psychological aspects of psoriasis can induce more remissions than ointments do. Group work with psoriasis patients indicates that improved responses can be obtained by discussion of the problem in the community (Coles, 1965; 1967), but psycho-social approaches are tremendously time-consuming and rate 1-1 doctor/patient relationships are impossible with the number of cases involved. The psycho-social environment of the deaf has been improved by otolaryngologists paying attention to their community needs. Neurologists are interested in spastics and epileptics. Diabetics and patients on renal dialysis are assisted socially by physicians. Has the skin patient been equivalently improved by dermatologists?

Only in the field of industrial dermatitis have dermatologists put much effort into the improvement of the social and economic working environment of the patient. Today the community's role in monitoring the health service is but one of the many changes in relationship between the medical profession and the patient. There are clear directives in 'the Grey Book' to explore the needs of the community and to be concerned not merely with the established bodies and institutions concerned in health care, but also with the process of being ill. There is a need to delineate where and when skin disease is interfering with normal living and to encourage self and community care so that some of the relevant load is taken away from the established services. Perhaps if social and economic strains were removed, the diseases themselves might melt away. We submit that the dermatologist is in risk of being left behind or on one side in this respect. It would be a disaster if patient groups or consumer organisations were to become anti-dermatologist. This could happen if the profession did not look more closely at the needs and demands of patients and their advisers.

Dermatologists have a better chance than almost any other branch of the profession in proving their value to the community as educators of other doctors and the public and as advisers on problems within the community (Caplan, 1973). In this field of medicine they could take the lead. Few specialists are listened to with such interest as can be seen from the large audiences that attend well-prepared lectures on the skin, but optimal conditions of patient care in the community include properly equipped and staffed skin units which are open at all times to patients and their doctors. Are we in dermatology pressing hard enough for units of this kind?

Some aspects of patient management and education cannot be the sole prerogative of dermatologists.

It is becoming increasingly apparent that patient associations such as the Psoriasis Association can effect changes in the environment that dermatologists are by themselves unable to achieve, but that such changes are most effective where the two sides can work together in partnership. The Psoriasis Association has, for example, effected changes at a national level through the lobbying of the Hairdressing Council, the Amateur Swimming Association and the Institute of Baths Management. Its publicity of the term 'psoriasis as a non-infectious disease' has already lightened the burden of many patients. A lecture by a dermatologist to local hairdressers can do more for the scalps of the local population than any amount of lotions prescribed through the NHS. The patient's own general practitioner himself needs to be better educated and the provision of 3 months' uninterrupted training in dermatology for doctors about to enter the local service has already greatly improved the standard of patient care in the Oxford area. Education of general practitioners, nurses and social workers can do far more to help the patient than any prescription by a consultant. Patients, too, of course need education. One of us (RBC) offers 1 or 2 hours of such care to all new patients with psoriasis. The consultant must think of the patient practitioner relationship right from the start; but where 'whole person' medicine is known to be being carried out by a general practitioner, he can confine himself to technical matters relative to his specialty. The medical transference situation can reside with the practitioner who can carry out treatment by relationship and casework in the mode he has developed for himself (Coles & Bridger, 1969).

It is well known that many severe psoriatics learn to put up with the condition and have some sort of life with it. It is not easy in a busy outpatients department to help patients to orientate themselves towards this better existence. It may be simpler to use strong treatments when perhaps 'orientation' and community support would be more valuable, more constructive and more economically acceptable. The Psoriasis Association is pioneering hopefully, beginning in 1975, an integrated programme of research into mechanisms and questionnaires, which are in some sense therapeutic. Perhaps more important is the aim of the Association to establish the nature and extent of the impact of the disease on patients, their families and the community, including the medical care services, from an economic viewpoint and to establish whether a programme designed to alleviate the impact of the disease is effective in so doing and makes better use of limited resources than alternative health programmes. It is hoped to analyse the psycho–social environment of psoriatics consequent upon the making of the diagnosis and the treatment of the condition and it is hoped to detect ways in which psoriatics cope in their environment and to shed light on the stigma and social disability consequent upon the illness. Another part of the programme is the establishment of the most effective use of the media to inform all five social classes of the public about psoriasis. Under the control of a National Committee and three sub-committees, particularly a Medical and Research sub-committee, the full-time Director and two secretaries answer several thousand letters a year for the Association and clearly fulfil a social need not otherwise dealt with by dermatologists. At the same time the Association takes pains not to interfere with the relationship between the patient and the doctor and never gives treatment advice. At a local level, active branches have already helped dermatologists and effected improvements in local services which unsupported demands by specialists alone could not have achieved. Patient associations can approach the press and such bodies as community health councils without fear of ethics or advertisements and have already done so. Their local branches have registered themselves at charity fairs and have organized hospital fêtes.

Such activities are helpful in establishing the role of dermatology in the community and in lightening the social burden of the skin patient, but specialist support is always necessary.

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Book Review

Dermatophytes in human skin, hair and nails. JAMES T. SINSKI (1974) Springfield, Illinois: Charles C. Thomas. Pp. 57, Illustrations (black and white) 35. Price £4.00.

Dr Sinski uses the term dermatophytes more broadly than is usual and he explains in the introduction that this volume is offered as a 'photographic essay . . . concerned with the typical as well as atypical morphology of fungi in human skin, nails and hair'. Most readers of this journal would no doubt agree that a small, well illustrated introductory text on the subject of direct microscopy of the superficial mycoses would be of great value in the training of clinical dermatologists and of laboratory workers. The usefulness of such a book to more experienced readers would certainly be enhanced by comprehensive coverage of the rarities.

The author of this volume has not attempted the latter aim and it must be judged entirely on its value for the post-graduate student, new to the craft of direct microscopy. Unfortunately even within this more limited compass it is less than satisfactory. One or two examples of its short-comings will suffice to explain why. Dr Sinski mentions that Parker Quink blue-black ink is a useful addition to potassium hydroxide but fails to mention that it stains different fungi at differing rates, being quick for pityrosporum species and slow for candida and the dermatophytes. His account of tape sampling would not enable a newcomer to produce satisfactory results for himself and he does not even mention the use of adhesive slides in skin surface biopsy. The 35 black and white photo-micrographs are all printed two to a page. Each has a commentary but these notes are rather pedestrian and fail to bring out general principles. For example the author does not make it clear that the presence of arthrospores in a length of mycelium immediately suggests a dermatophyte infection and excludes candidiasis. When dealing with yeasts he is anxious to stress that in candida infections the yeasts may be compressed together giving the appearance of a mosaic, whereas in Malassezia furfur this does not happen. He does not trouble to mention that candida has a thin cell wall, and pityrosporum species one of a very thick and rigid construction, surely the likely explanation for their different appearances. In the artefact section notable omissions are photographs of the sweat duct coiling through the stratum corneum and the spherical globules of Parker ink, which sometimes settle out and which can look very like stained yeasts to the untutored eye.

This slender volume cannot be recommended. Indeed its very size is deceptive. Within its 64 bound pages there is the equivalent of 21 pages of blank paper. This calculation does not include the inevitable wastage on the pages where titles, catalogue numbers and contents are set out. Does a book of this size really need a general introduction from the Editor of the series (1½ pages)? Surely not. Some of the 35 photomicrographs could have been trimmed without loss of content and would then have needed much less than the 17½ pages allocated to them. In Rebell and Taplin, 'The Dermatophytes' 1970 Edition, University of Miami Press, 35 rather similar illustrations are grouped together and take up space equivalent to 3 pages of this volume. Charles C. Thomas please take note, there is no place in 1975 for expensive books as flabby as this one, no matter how slim they appear.

S.O.B. ROBERTS

British Journal of Dermatology (1975) **93**, 115.

Microbiology Review

Immunosuppression and skin infection

Immunosuppression is a state in which established immunity and the response to new antigens are impaired (Bagshawe, 1972). The term covers changes in cell-mediated as well as in antibody-mediated immunity. The immunosuppressed state can have many causes ranging from spontaneous illness, particularly diseases of the lymphoreticular system, to the deliberate use of immunosuppression, for example to counter graft rejection. It is not easy to separate the complications of immunosuppression from those of bone marrow suppression, for most immunosuppressive drugs also damage marrow cells; indeed of the immunosuppressive drugs only the steroid hormones seem not to be myelotoxic (Kay & Jameson, 1970).

The recent increase in the clinical use of immunosuppression means that dermatologists can expect to be shown patients suffering from common infective conditions in an unusual form, or even from previously very rare infections. Most immunosuppressive agents lack selectivity, and interfere, to a variable degree, with both cellular and humoral immunity; but if a generalization is possible, it is that most of the case reports have been of viral, fungal and Gram-negative infections, suggesting that the main effect has been on cell-mediated immunity.

Severe, often fatal, infections are by now a well known hazard: for example, in renal transplant patients (Rifkind *et al.*, 1964 a, b, 1967b; Myerowitz, Medeiros & O'Brien, 1972; Duma, 1972), in leukaemic patients (Baker, 1962), and in other conditions (Reynold, Walls & Pfeiffer, 1966; Fagundes, Busato & Brentano, 1971; Gurwith, Stinson & Remington, 1971; Nirmul *et al.*, 1971; Young *et al.*, 1974). However it is with instances of skin disease that we are concerned here and these must be seen in perspective against the background of other infections. In one study on infection after renal transplantation (Bach *et al.*, 1973) involving fifty-one recipients of grafts, fifteen developed infections as complications, and of these, only two had skin infections, both also having infections of the lung with nocardia and aspergillus. Skin infection (22%) was less common than lung (42%) or urinary tract (35%) infection in another series of 216 patients with renal transplants (Eikhoff *et al.*, 1972). Turcotte (1972) looked for serious infections in ninety-three patients at risk for a total of 1000 months after transplant; among the serious infections were herpes simplex (6), cellulitis (4), and herpes zoster (3). Sometimes the features of the infection make diagnosis difficult. For example, of three patients described with skin infections during immunosuppressive therapy (Park, Goltz & Carey, 1967), one had persistent varicella, one an extensive, non-healing herpes simplex, and one a disseminated histoplasmosis with a widespread erysipelas-like rash.

Viral infection. Herpes simplex after renal transplant may be unusually extensive, haemorrhagic and persistent; in four affected patients (Montgomerie *et al.*, 1969) the herpetic infection caused the death of one individual and was an important factor in the death of the other three. Severe herpes simplex has also been seen in patients with lymphomata (Lynfield, Farhangi & Runnels, 1969). The well known susceptibility of patients with atopic eczema to herpes simplex and vaccinial infections, and now apparently to dermatophyte infections (Jones, Reinhardt & Rinaldi, 1974) may also relate to abnormal cell-mediated immunity. Experimentally, herpes simplex can be recalled in mice by the administration of prednisone, though lesions do not reappear for 12–30 days (Underwood & Weed, 1974).

Other viruses also flourish. Viral warts may smother the skin of patients with Hodgkin's disease

(Smithers, 1967). Warts are common in Hodgkin's disease, with its reduced cellular immunity, but not in myeloma, an example of abnormal humoral immunity (Morison, 1974). It has even been suggested that the appearance of luxuriant warts should suggest the possibility of immune deficiency (Perry & Harman, 1974). Brodersen, Genner & Brodthagen (1974) in a study of tuberculin sensitivity found that children with warts had a mean zone of induration of 10 mm compared with about 15 mm in controls. They point out that it is not possible to decide whether the reduced sensitivity results from the wart infection or whether the warts are a marker for a defective cellular immunity. Molluscum contagiosum has been recorded in two patients treated with methotrexate and prednisone (Rosenburg & Yusk, 1970), each patient having about 600 lesions.

Varicella was reported in a child treated with azathioprine (Fine *et al.*, 1969) and Armstrong and his colleagues (1970) were able to relate the death of cancer patients who developed varicella to a failure to produce high levels of interferon; three patients were leukopenic, and two died from the infection; while four other patients who were not leukopenic produced high levels of interferon and all recovered. An association between herpes zoster and Hodgkin's disease or leukaemia has been reported by Muller (1967) amongst others.

Cytomegalovirus seems also to be an important cause of infection (Kanich & Craighead, 1966; Rifkind, Goodman & Hill, 1967a), particularly of the eyes of immunologically compromised patients (Porter *et al.*, 1972). Renal transplant patients also show an increased excretion of the *Epstein–Barr* virus (Strauch *et al.*, 1974). A giant orf lesion has been described in a patient receiving immunosuppressive agents (Savage & Black, 1972). Severe, progressive vaccinia may develop in those persons with inadequate levels of circulating antibody (hypogammaglobulinaemia) and also in those with a deficient delayed hypersensitivity response. Such patients may include those with thymic alymphoplasia, leukaemia, Hodgkin's disease and those on immunosuppressive and corticosteroid drugs (Neff & Lane, 1970; Copeman & Banatvala, 1971).

Finally, there is a possibility that the high incidence of tumours of the lympho-reticular system which is found in immunosuppressed patients may be due to the uncontrolled proliferation of oncogenic viruses (Allison, 1970).

Fungal infections. Unusual fungi have been particularly troublesome in some patients. *Fusarium solani* has been reported as complicating treatment for acute leukaemia (Cho *et al.*, 1973); this was the first recorded case of disseminated infection with this fungus. Infections with *Fusarium* are rare but do occur in patients not on immunosuppressive therapy (Hardy, 1974). A *Phoma* species caused a subcutaneous abscess in an immunosuppressed patient (Young, Kwon-chung & Freeman, 1973). Primary cutaneous aspergillosis has been recorded in the absence of chemotherapy (Cahill, El Mofty & Kawaguchi, 1967) but could be increased in compromised patients in the same way that lung infections become especially common (Gowing & Hamlin, 1960; Gurwith *et al.*, 1971; Stinson et al., 1971; Burton *et al.*, 1972; Burton & Greenough, 1973). Roberts (1969) reported that systemic corticosteroid therapy was associated with an increased susceptibility to pityriasis versicolor. It may be significant that three of six seriously ill patients, in a total of twenty-five new patients, were receiving azathioprine, two for renal disease. The association of candidiasis with systemic steroids is too well known to need much comment here. Skin lesions may appear in neutropenic patients who have Candida septicaemia but do so only in those with an easily demonstrable blood stream infection (Bodey & Luna, 1974). Diffuse severe thrombocytopenic purpura has been reported in two patients with histoplasmosis; transfer from the first patient to the second appeared to be due to the renal transplant (Hood *et al.*, 1965).

Bacterial infections. M. tuberculosis may become disseminated during immunosuppression (Neff &

Hudgel, 1973; Lakshminarayan & Sahn, 1973). The prevalence and severity of tuberculosis in 201 patients with various neoplastic diseases were reviewed by Kaplan, Armstrong & Rosen (1974). Patients receiving the most vigorous therapy more often developed severe tuberculosis than those receiving only radiation treatment. The prevalence of tuberculosis was highest in lung cancer and lymphoproliferative disorders and lowest in those with cancer of the gut or abdominal organs. One boy with Hodgkin's disease and tuberculosis showed a curious histological picture, like that of lepromatous leprosy (Skinsnes, 1968). *M. marinum* infections can be exacerbated by corticosteroid therapy alone (Aaronson & Park, 1974), and have been reported as mimicking *M. ulcerans* infection in a patient with lymphosarcoma (Sage & Derrington, 1973). Two renal transplant patients developed multiple subcutaneous abscesses due to *M. cheloni (abscessus)* (Graybill *et al.*, 1974). Skin ulcers may be infected with atypical mycobacteria and 'non-pathogenic' fungi as a complication of immunosuppressive therapy (Lomvardis & Madge, 1972).

Toxic epidermal necrolysis (TEN) related to bacterial infections is usually confined to young children, though it has been seen in adults (Rothenberg *et al.*, 1973). There are now several case reports of this condition developing in adults with altered immunity. One patient developed TEN during a graft-versus-host reaction (Peck, Herzig & Elias, 1972). The patient yielded *Ps. aeruginosa* from lesions in the last week of life but it seems likely that this was merely due to faecal contamination. There have been other reports of staphylococcal TEN in adults: for example with deficient cell mediated immunity (Reid, Weston & Humbert, 1974); in a patient receiving prednisolone and azathioprine (Levine & Norden, 1972); in a patient with leukaemia (Hawley & Aronson, 1973; Norden & Mendelow, 1974); during renal failure (Hunter & Davison, 1973); in lymphoma patients (Caldwell, Montgomery & Peachey, 1967); and in heroin abuse (Lewis, 1974). Prednisolone and azathioprine have been shown to potentiate the appearance of TEN in baby mice challenged with phage group II *Staph aureus*; though no effect could be demonstrated on the action of the epidermolytic toxin alone (Wiley *et al.*, 1974) and it seems clear that it is host resistance to the organisms which is impaired by the immunosuppressive drugs. TEN also occurs in the apparent absence of staphylococci in patients with graft-versus-host reactions (Ammann & Tooley, 1972).

About 10% of patients suffering from pseudomonas septicaemia during cancer therapy are said to show skin lesions such as blebs, cellulitis, and ecthyma gangrenosum; the portal of entry to the organisms causing the septicaemia was thought to be other skin lesions in 30% of the patients (Forkner *et al.*, 1958; Fishman & Armstrong, 1972; Peck, Elias & Graw, 1972). *Escherichia coli* septicaemia may cause toxic epidermal necrolysis (Plant & Mirani, 1972) or sub-epidermal bullae (Fisher, Berger & Keusch, 1974).

It is noticeable how often renal failure figures in these reports, including the patient who developed Norwegian scabies while on immunosuppression (Paterson, Allen & Beveridge, 1973). An additional suppressive mechanism may be the inhibition of lymphocyte function caused by methyl guanidine which is present in high concentration in the plasma of uraemic patients (Harris *et al.*, 1962).

Nothing seems to be known of the skin flora of patients suffering graft rejection, or who are acutely ill and receiving high doses of immunosuppressive agents, though those who are well maintained have a skin flora very like that of normal persons (Noble, Rebel & Smith, 1974). It may be that many of the organisms are from the patients own, endogenous, gut flora, yet it is these organisms that proliferate on the skin of severely ill patients (Gallus, Stratford & Dixson, 1969). We may speculate that in some diseases associated with immunosuppression, the skin may be the portal of entry for pathogens; indeed, Spiers (1974) reporting death from septicaemia in three of four patients with leukaemia or lymphoma who had initially been merely colonized with *Pseudomonas aeruginosa* suggested that 'Isolation of pseudomonads from any cutaneous lesion, however minor, is an . . . indication for systemic therapy.'

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kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
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British Journal of Dermatology, Volume 93, Number 1, July 1975

CONTENTS

PAGE

Clinical and Laboratory Investigations

Lysosomal Hydrolases of the Epidermis. 1. Glycosidases 1
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK

Prealbumin Bearing Lymphocytes as an Indicator of Hapten Protein Carrier in Certain Varieties of Dermatitis 11
S. HUSZ, F. HAMERLINCK AND R. H. CORMANE

The Diagnostic Significance of Immunoglobulin and Fibrin Deposition in Lichen Planus 17
E. ABELL, D. G. C. PRESBURY, R. MARKS AND D. RAMNARAIN

Deficiency of Circulating IgA and IgM in Adult Patients with Lichen Planus 25
L. STANKLER

The Effects of Catecholamine and Related Compounds on the Adenyl Cyclase System in the Epidermis 29
KUNIHIKO YOSHIKAWA, KENJI ADACHI, KENNETH M. HALPRIN AND VICTOR LEVINE

The Effect of Treatment on Plasma Uroporphyrin Levels in Cutaneous Hepatic Porphyria 37
B. R. ALLEN, SHEENA PARKER, G. G. THOMPSON, M. R. MOORE, F. J. DARBY AND J. A. A. HUNTER

Repeated Tissue Sampling with a Dental Broach. A Trial in Cutaneous Leishmaniasis 43
W. A. D. GRIFFITHS AND W. DUTZ

The Percutaneous Penetration of Nandrolone Decanoate 47
M. I. FOREMAN AND I. CLANACHAN

Secondary Syphilis: a Clinico-pathological Review 53
E. ABELL, R. MARKS AND E. WILSON JONES

Rosacea of Common Male Baldness 63
MALGORZATA GAJEWSKA

Pharmacology and Treatment

Evaluation of Coal Tar Fractions for Use in Psoriasiform Diseases Using the Mouse Tail Test. III. High Boiling Tar Oil Acids 67
ROSANNE WRENCH AND ADAM Z. BRITTEN

Evaluation of Dithranol and a 'Synthetic Tar' as Anti-psoriatic Treatments Using the Mouse Tail Test 75
ROSANNE WRENCH AND ADAM Z. BRITTEN

Efficacy of Miconazole in the Topical Treatment of Tinea Pedis in Sportsmen 79
J. C. GENTLES, G. R. JONES AND D. T. ROBERTS

Granuloma Annulare and Necrobiosis Lipoidica Treated by Jet Injector 85
G. SPARROW AND E. ABELL

Case Reports

Pseudoxanthomatous Mastocytosis 91
W. A. D. GRIFFITHS AND K. DANESHBOD

Urticaria and Hyperthyroidism 97
J. L. PACE AND MAURICE GARRETTS

Recurrent Necrosis of the Skin and the Mouth 101
NABIL I. ELSAHY

Methotrexate Pneumonitis in a Psoriatic 107
E. FROM

Comment

The Psoriasis Sufferer in the Community 111
R. B. COLES AND T. J. RYAN

Book Review 113

Review Article

Immunosuppression and Skin Infection 115
J. A. SAVIN AND W. C. NOBLE

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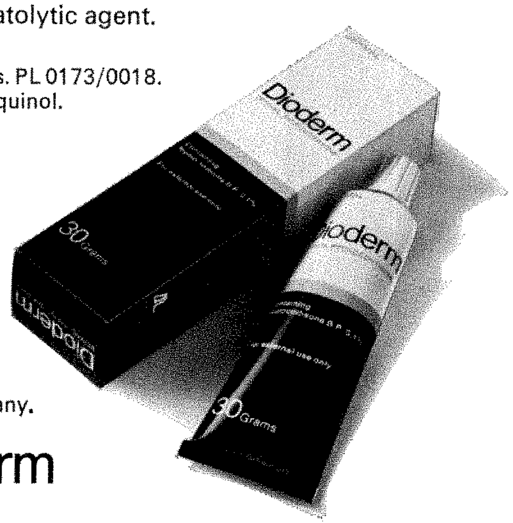

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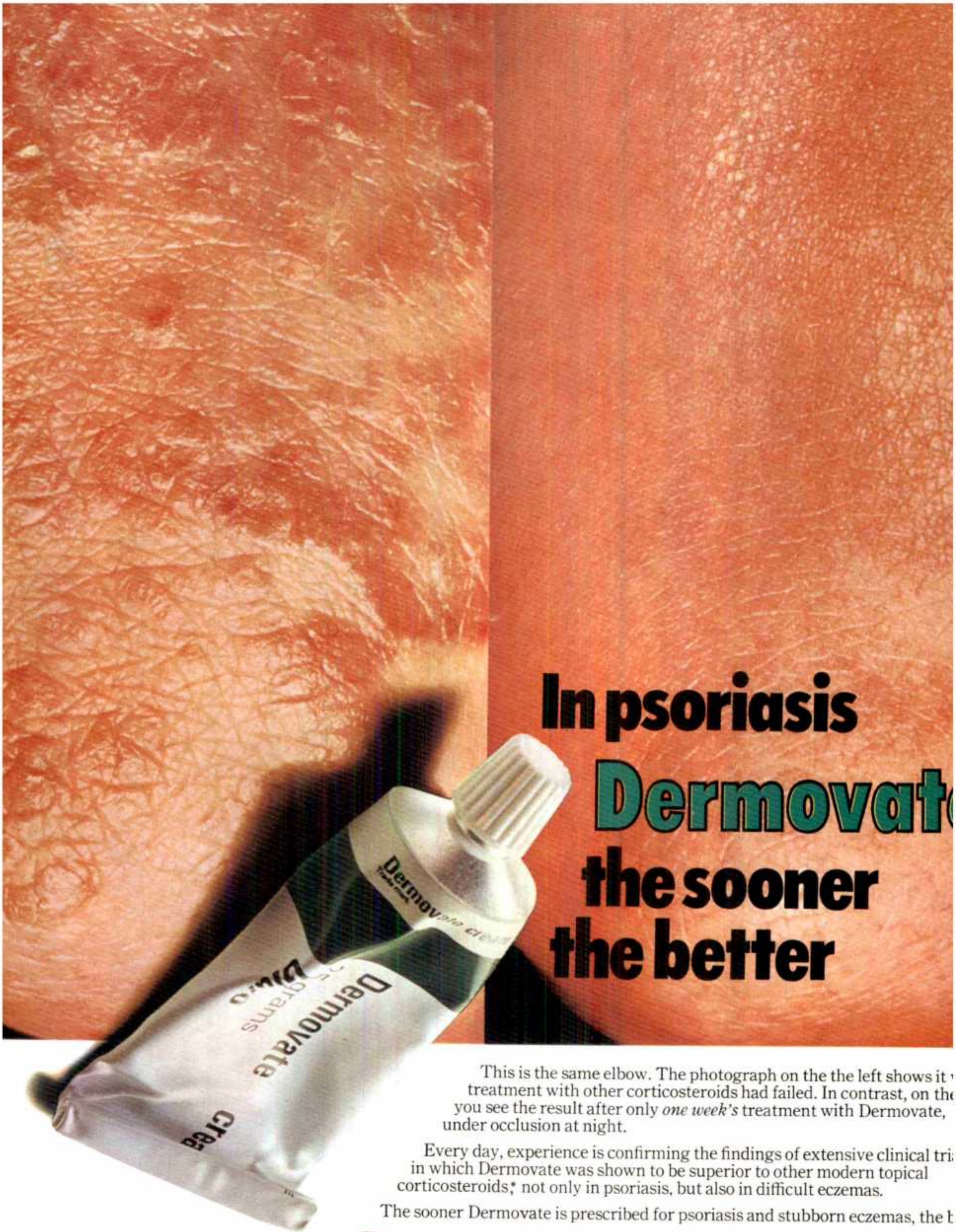

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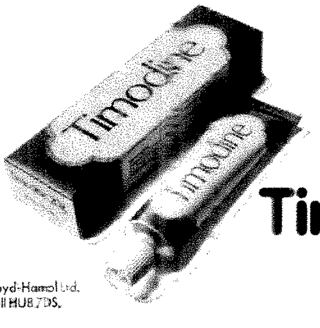

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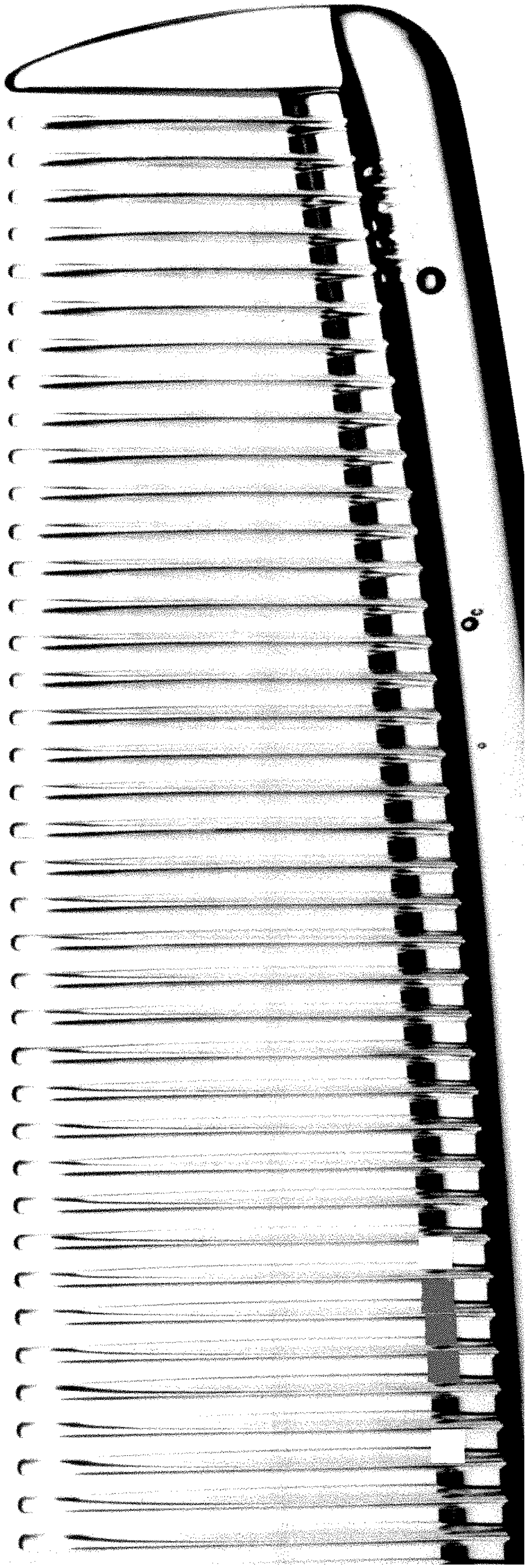

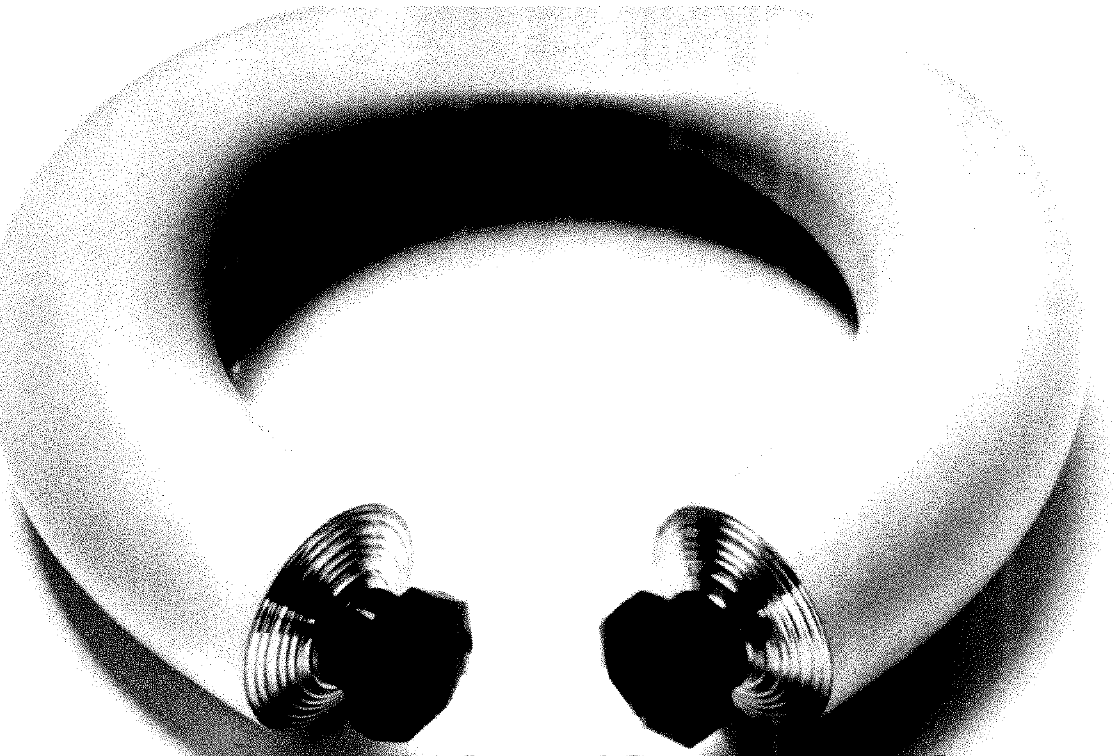

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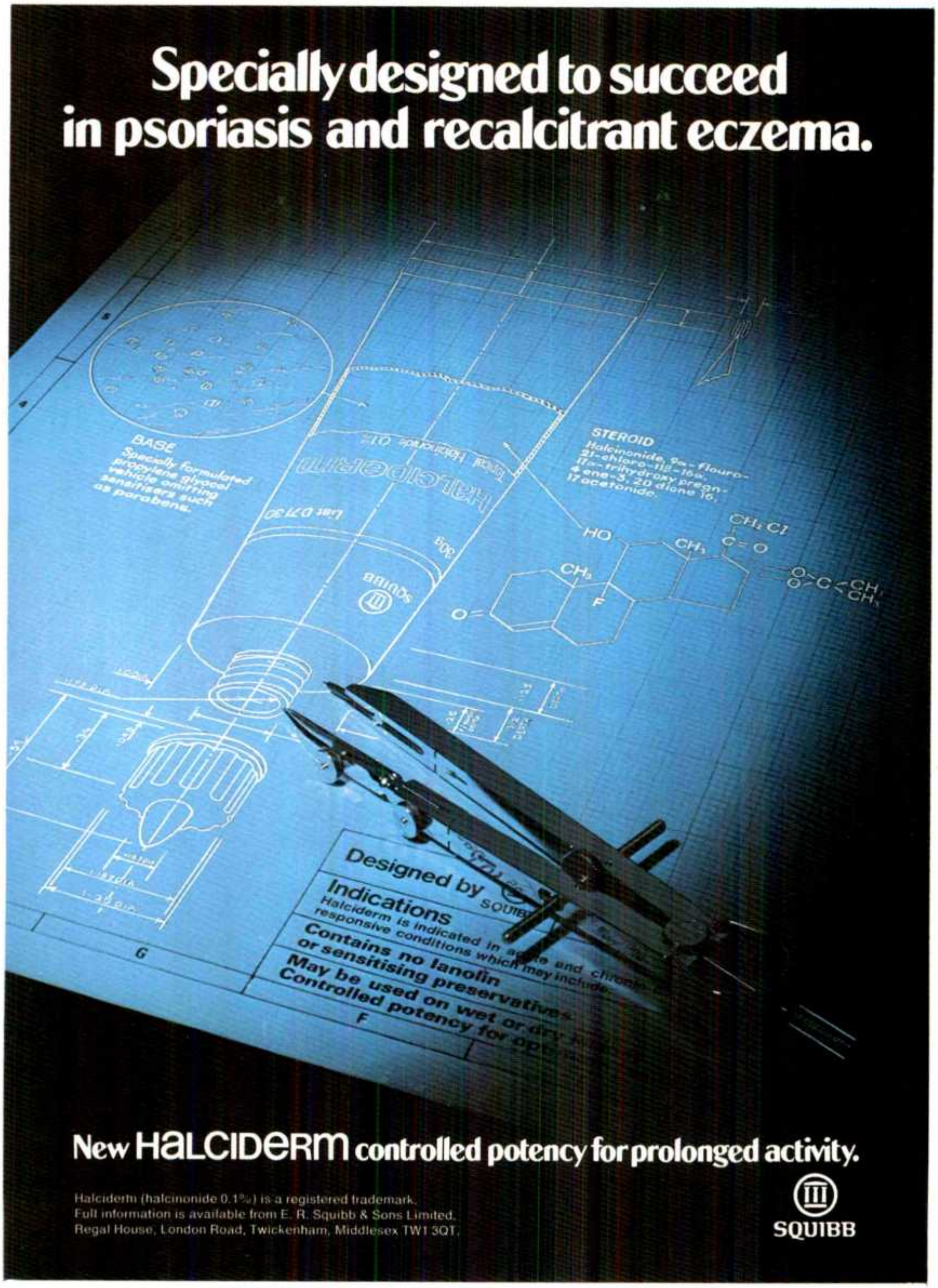
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Index Vol. 150, No. 1, 1975

Preface to the 150th Volume of 'Dermatologica'

Lapière, C.M. (Liège): The Gap. Avant propos to the editorial article

Editorial

Kefalides, N.A. (Philadelphia, Pa.): Basement Membranes: Current Concepts of Structure and Synthesis

Baker, H. (London): Psoriasis: A Review. Part I

Notowicz, A.; Menke, H.E.; Stolz, E., and Vuzevski, V.D. (Rotterdam): Solitary Papular Lesions on the Penis in Insufficiently Treated early Syphilis. A Report of Two Cases

Yesudian, P. and Thambiah, A. (Madras): Familial Syringoma

Caro, I.; Sher, M.A., and Rippey, J.J. (Johannesburg): Pseudoxanthoma elasticum and Elastosis perforans serpiginosa. Report of Two Cases

Cotton, D.W.K. and Rossum, Elisabeth van (Nijmegen): Screening for 6-Phosphogluconate Dehydrogenase Abnormalities in the Blood of Dermatological Patients

Kuni, Ch.C. (Denver, Colo.): Lack of Effect of Vitamin A on Differentiation in Squamous Carcinoma

Vloten, W.A. van and Polano, M.K. (Leiden): Bleomycin Therapy in Mycosis fungoides

The Interesting Case · Der interessante Fall · Un cas intéressant

Korting, G.W. (Mainz): Ueber Porphyria-cutanea-tarda-artige Hautveränderungen bei Langzeithämodialysepatienten
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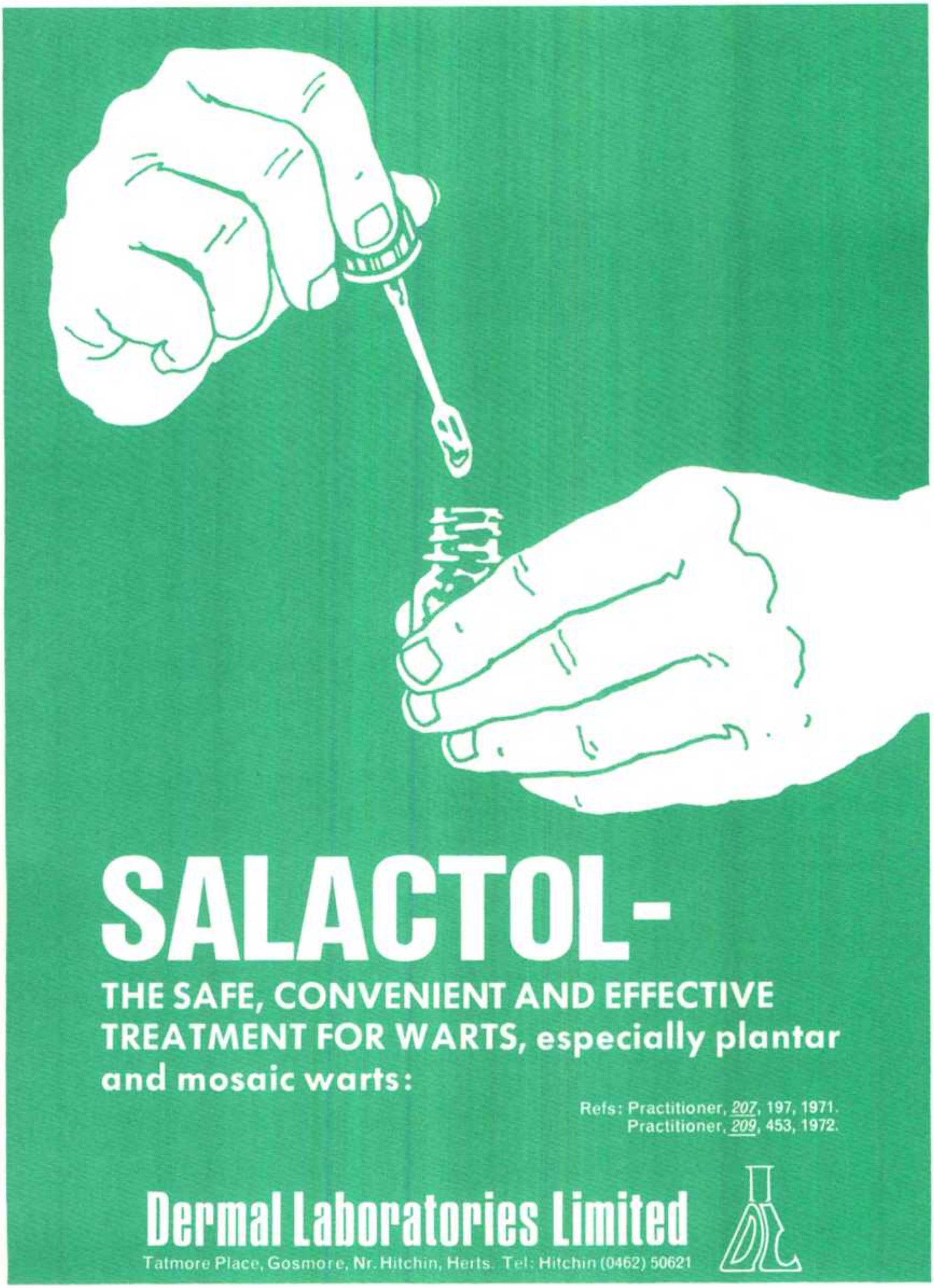

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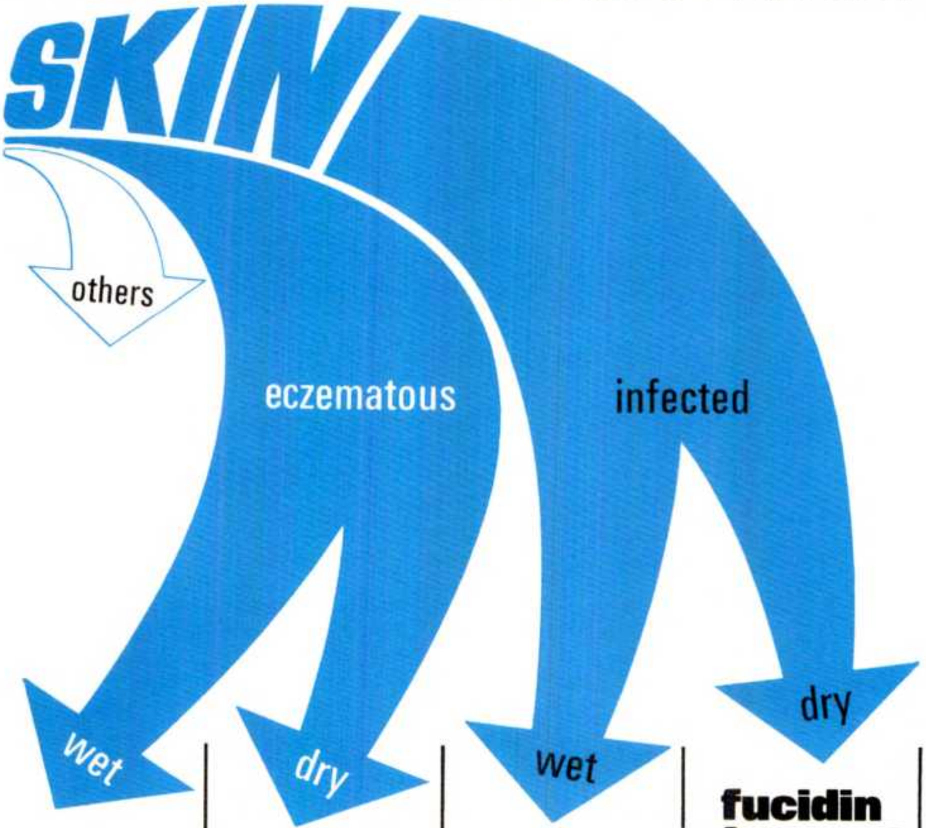

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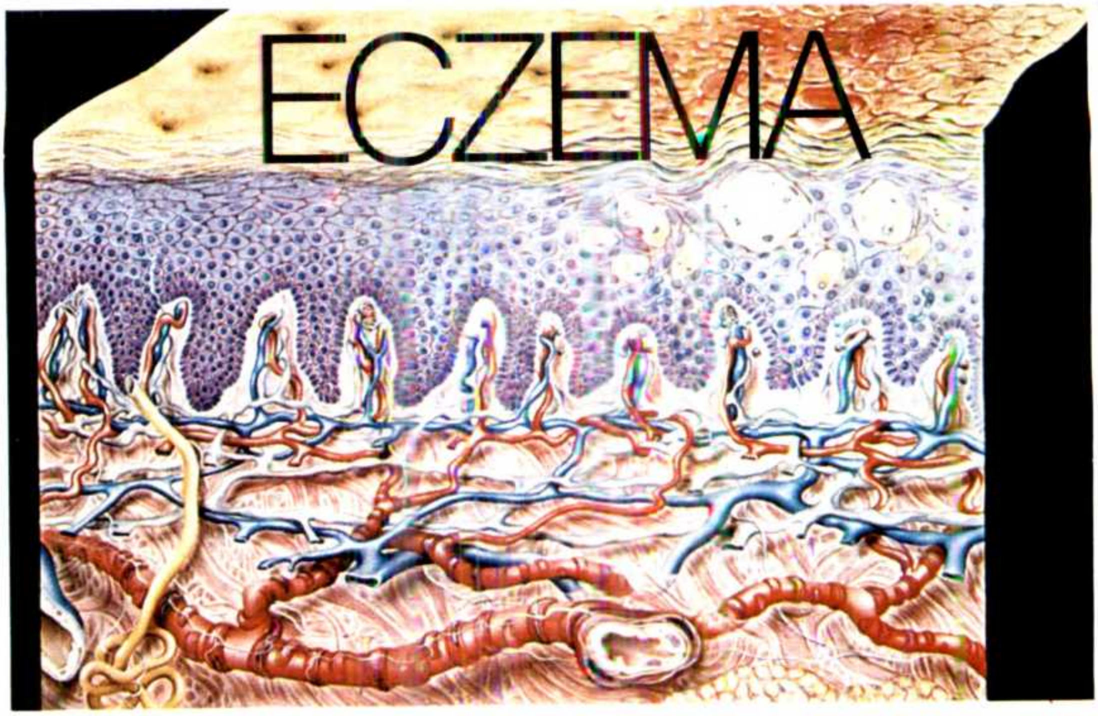

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Clinical and Laboratory Investigations

Naevus superficialis lipomatosus

A CLINICOPATHOLOGICAL REPORT OF TWENTY CASES

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D.PONGSEHIRUN†

Department of Dermatopathology, The Institute of Dermatology, St John's Hospital for Diseases of the
Skin, Lisle Street, Leicester Square, London, WC2H 7BJ

Accepted for publication 14 November 1974

SUMMARY

Naevus superficialis lipomatosus (NSL) is a rare malformation consisting of multiple or solitary papules usually occurring on the lower trunk or gluteal regions. In this study the clinical features of twenty new cases are described with a detailed account of the histopathology. Notable were the marked variability in the amount of ectopic fat found in the lesions, and the presence of associated abnormalities of the connective tissue, the vessels, and the skin appendages. In early lesions, mononuclear cells differentiating into lipoblasts could be identified around proliferating capillaries.

Two clinical types were distinguished. First, the classical type (7 cases) consisting of multiple lesions occurring in a zonal distribution, and secondly the solitary papule or nodule (13 cases). The latter type can be difficult to distinguish from a skin tag with fatty herniation.

Judging by the paucity of case reports, naevus superficialis lipomatosus (NSL) must either be extremely uncommon or be unrecognized by most clinicians who see it. In fact, we have been able to trace only two brief case reports of patients who have been demonstrated at dermatological meetings in this country (Colin-Jones & Calnan, 1970; Shrank, 1967).

Following Hoffmann & Zurhelle's original case report in 1921 describing multiple lesions present since birth on the left buttock of a 25-year-old man, there were no further cases reported until that of Robinson & Ellis in 1937. We have been able to find records of only forty acceptable instances of NSL in the literature,‡ and this includes a number of examples cited by Kurihara *et al.* in 1967 from Japan.

It is not a dramatic disorder, and most patients state that their lesions have either been present at birth or have developed within the first two decades of life. In most reports the naevi presented as soft, multiple, skin-coloured or yellowish papules, sometimes coalescing into plaques with a cerebriform surface. Characteristically the papules are distributed on one buttock, but they may overlap onto the adjacent skin of the back or the upper thigh. However, naevi have been recorded that were confined

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Present addresses: * Welsh National School of Medicine, Cardiff, Wales. † Institute of Dermatology, Bangkok 4, Thailand.

‡ Four more instances have recently been reported by Hönigsmann and Gschnait (*Z. Hautkr.* (1974) **49**, 517).

to the upper thighs (Nikolowski, 1950; Blumenthal, 1955; Holtz, 1955), to the lumbar region (Hering, 1956; Shroff *et al.*, 1971) or to the thorax (Kúta, 1951; Solente & Harter, 1956; Alkiewicz, 1959). Other affected areas have been over the greater trochanter (Thöne, 1951; Szodoray & Szondy, 1961) and the abdominal wall (Abel & Dougherty, 1962). Usually the lesions do not cross the midline, but bilateral involvement affecting the opposing surfaces of the buttocks and the coccyx was reported by Robinson & Ellis (1937). Nikolowski (1950) reported four instances of solitary lesions of NSL and Kurihara *et al.* (1967) mentioned two other cases in the Japanese literature. Solitary lesions may occur at unusual sites, and naevi of the ear and of the scalp respectively were described by Knoth (1962) and Weitzner (1968).

Usually there are no associated abnormalities, and we consider that the patients reported as NSL with skeletal and other anomalies (Miedziński, 1963; Stollmann & Oehlschlaegel, 1969) were probably examples of the Goltz syndrome (focal dermal hypoplasia). However, two genuine examples of NSL have been described with co-existent pigmentary anomalies. Pierini, Abulafia & Lebedinsky (1970) recorded café-au-lait macules in their patient, and scattered leukodermic spots were noted in the case of Robinson & Ellis (1937). Increased hairiness was reported over the naevus in the recently described patient of Finley & Musso (1972). The lesion of NSL, once formed, usually remains static, although it has been reported that new lobules may continue to develop over the course of many years (Cabre, Lasanta & Fuertes, 1971).

Until this report, the largest series of cases in the literature has included six patients only (Nikolowski, 1950), and in view of the comparative rarity of NSL the purpose of this paper is to give details of a further twenty patients, and to discuss the histogenesis of the disorder.

Clinical data

The twenty cases we shall describe were encountered as follows: Fourteen of the patients were attending St John's Hospital Out-Patients Department at the time of the diagnosis (1960–1973).

Four patients were referred to us by other dermatologists (Drs Colin-Jones, Dowling Munro, Du Vivier and John Morgan.)

Specimens from two additional cases had been sent for histological diagnosis from other Pathology

TABLE I. Sex and age of patients by decade at time of diagnosis

10–19	20–29	30–39	40–49	50–59	60–69
●♂ ●♂	●♂ ●♂	○♀ ○♂	●♀ ○♂	○♀ ○♀	○♀
	○♂ ●♀		○♀ ○♂	○♂	
	●♀ ○♀		○♂		
	○♀				

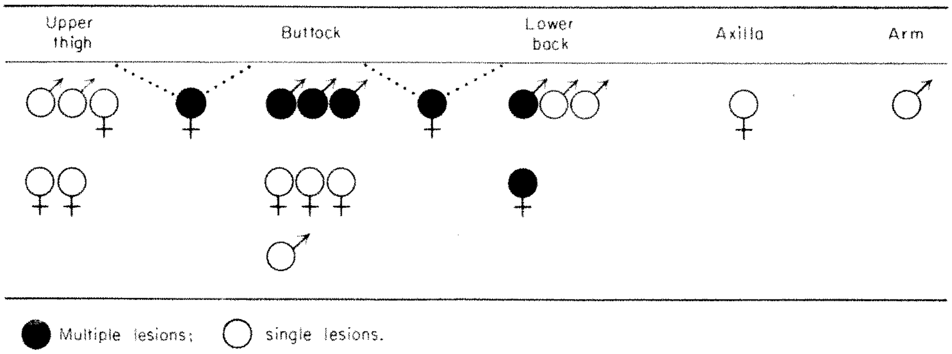
● Multiple lesions; ○ single lesion.

M,10; F,10; av. age, 36 (14–64).

Departments by Drs J.Mullaney and H.K.Heffernan. At least one adequate biopsy was available for study from each patient. Our report includes the two patients previously reported by Colin-Jones & Calnan (1970) and Shrank (1967).

Ten patients were male and ten were female. The average age at presentation was 36 years (range 14–64). Table 1 gives sex and age of the patients by decade at the time of diagnosis. It can be seen that patients with the multiple lesions tended to be younger than those with solitary naevi. Seven patients had multiple lesions, the remainder had solitary nodules or papules only. Of the patients with multiple lesions, two stated that lesions had been present at birth, three dated the appearance of their lesions at adolescence, and the remaining two had developed their lesions in their mid-twenties. In patients with solitary naevi, all were over the age of 20 years at the time that their lesions were first noted, and some of these naevi had made their appearance as late as the fourth or fifth decade. The usual history was that the lesions were insidious in onset, symptomless and very slow-growing. The buttock or the closely adjacent skin was the most common site (Table 2).

TABLE 2. Site of lesions



The clinical diagnosis had not been suspected prior to biopsy in the great majority of the patients, and in none of those with only solitary naevi. The wide spectrum of pre-biopsy diagnoses is given in Table 3. It is apparent that the appearance of the solitary naevi was not distinctive; most commonly they were somewhat larger, and possessed a broader base than ordinary skin tags (achrochordon) (Fig. 1). They were usually flesh-coloured and of a soft consistency.

The seven patients with multiple lesions had clusters of variable-sized, soft, fleshy, elevated papules

TABLE 3. Suspected clinical diagnosis prior to biopsy

Solitary lesions		Multiple lesions*	
Accessory nipple	1	Warty naevus with comedones	2
Cyst	1	Multiple cellular naevi	2
Lipoma	1	Cobblestone naevus	1
Cellular naevus	3	Connective tissue naevus	1
Neurofibroma	1	Naevus sebaceus	1
Fibro-epithelial papilloma	3	Naevus lipomatosus	2
Sweat gland tumour	1	Multiple skin tags	1

* More than 1 possible diagnosis had been suggested in some instances at time of biopsy.

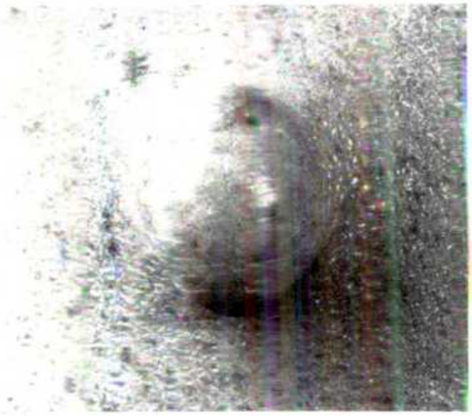


FIGURE 1. Solitary lesion on the right arm of a man aged 44 years. It had been present for 4 years.

in a zonal distribution across one side of the lower back or gluteal region (Figs 2 and 3). The individual papules were either irregularly scattered over the affected area, or a band-like or a zosteriform pattern was apparent (Fig. 3). The papules were either skin coloured or sometimes distinctly yellowish in tint. The surface was wrinkled rather than smooth, but not warty. Most of the papules were dome-shaped and sessile rather than papillomatous. In places the lesions coalesced to form plaques with a cerebriform outline. In two patients comedo-like plugs were present on the surface of many of the larger papules (Fig. 2). In one instance, recurrent boil-like lesions in the papules had caused a patient

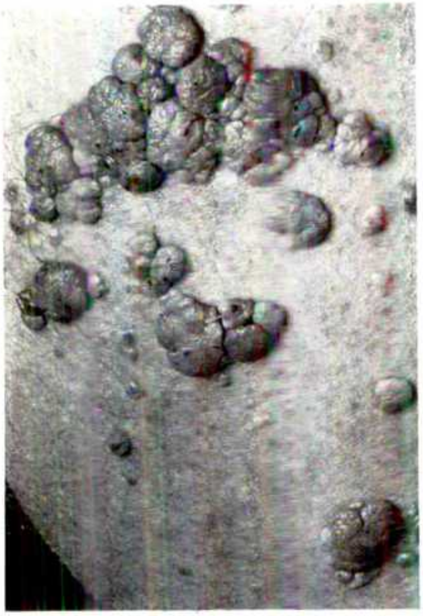


FIGURE 2. A man aged 21 years who had developed multiple papules and nodules over the right buttock since the age of 15 years.

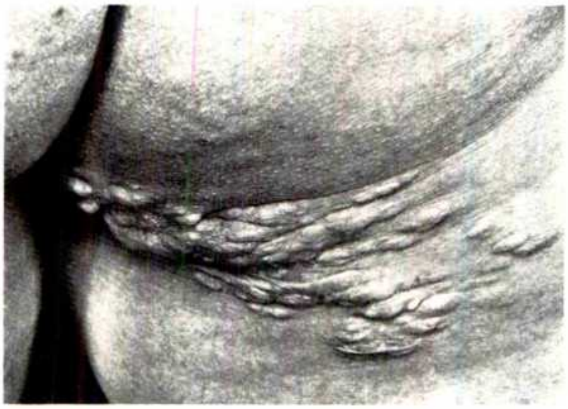


FIGURE 3. A woman aged 40 years with papules in zosteriform arrangement affecting the infragluteal fold and upper thigh.

to seek medical advice. None of the patients had any notable systemic illnesses, or record of other congenital defects.

Simple excision usually appeared to be adequate for solitary lesions if treatment was demanded; in one instance a solitary lesion of the right upper thigh in a man aged 57 years slowly recurred after an excision 5 years previously.

Histopathology

Twenty-six specimens from the twenty patients were available for study. Three biopsies were frozen and sectioned on a cryostat for subsequent histochemical examination. In at least one biopsy from each patient, groups of fat cells were present at the level of papillary dermis. This requirement for histological diagnosis we regarded as essential in order to separate a 'giant' skin tag with fatty herniation from NSL.

Our studies indicate that NSL is a complex connective tissue malformation in which the presence of ectopic fat tissue is but one component. Additional features are changes in the collagen and elastic fibres, small blood vessel proliferation and abnormality of the epidermis and the appendages.

Fat tissue

The amount of ectopic fat in the dermis varied greatly. In four specimens (three solitary and one multiple), 50% or more of the dermis was occupied by fatty tissue (Fig. 4). In about half of the specimens the misplaced fat accounted for 10% or less of the total bulk of the lesion. When small deposits of fat were present, the fat cells were situated in small foci around the subpapillary vessels (Fig. 5). Larger deposits of fat were less obviously related to the vasculature. The ectopic lipocytes were nearly all mature and of normal size. An occasional smaller, incompletely lipidized cell was observed, but in no instance were clusters of immature cells noted, of the kind found either in lipoblastomatosis or in brown fat. In those specimens with relatively large amounts of ectopic fat, the fat lobules were irregularly dispersed throughout the dermis (Fig. 4), and in this event the boundary between the dermis and the hypoderm was ill-defined or lost.

Connective tissue

It was clear in gross structure that excess connective tissue components were present in addition to

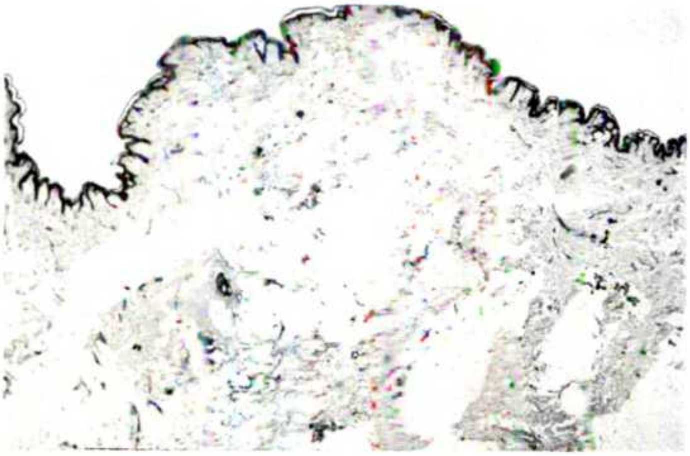


FIGURE 4. NSL. Extensive replacement of the dermis with lobules of ectopic fat. (From patient illustrated in Fig. 3) (H & E ×8).

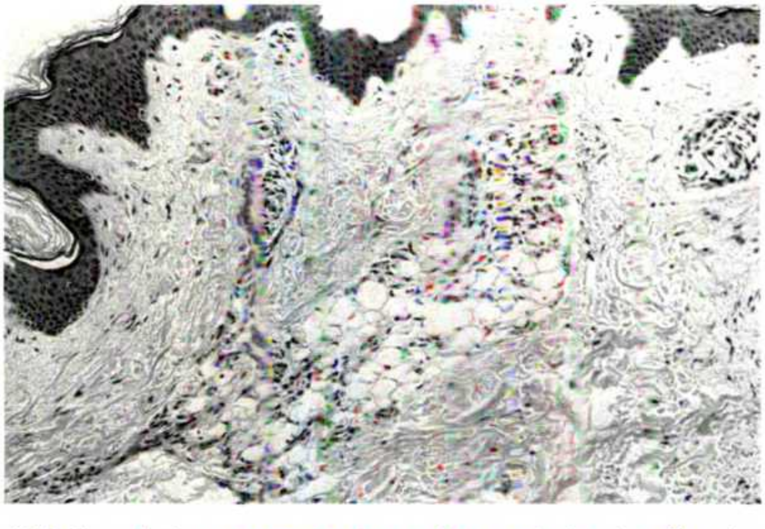


FIGURE 5. NSL. Fat cells sleeve tortuous vessels extending towards the subpapillary plexus. (From a man aged 21 years with multiple lesions) (H & E ×60).

ectopic fat, leading to the formation of elevated papules, and to complex papillomatous excresences with a cerebriform outline. In detailed structure, the connective tissue alterations were subtle. We formed the impression that the collagen was less compact than in normal skin, and the bundles were arranged more irregularly. The individual bundles did not appear abnormally coarse or thickened as in some other connective tissue naevi.

In many of the sections the tissue had a frayed appearance with a tendency to artefacts, which we interpreted as being due to loss of cohesion between dermal fibres resulting in their displacement by the knife in sectioning. The elastic tissue (orcein stain) usually appeared normal in the lower parts of the naevi, but there was a tendency for the fibres to be sparse towards the surface, particularly in the larger papules, and in places little of the subepidermal elastic felt remained. No gross changes in the elastic fibres of the kind seen in juvenile elastoma or elastofibroma dorsi were encountered. Mucin stains did not show a marked excess of ground substance. A small increase in mucin was limited to areas showing increased capillaries and active lipogenesis.

Vessel changes

In many of the specimens, the numbers of small blood vessels and capillaries were greatly increased in the subpapillary zone and the papillae (Fig. 6). The majority of these vessels were small, tortuous capillaries with narrow lumens, but some ectatic vessels were also observed. These capillaries were loosely cuffed by a variable number of spindle-shaped and rounded mononuclear cells and fat cells. There appeared to be an inverse relationship between the number of mononuclear cells and of mature fat cells sleeving the vessels. At high magnification, most of the mononuclear cells did not appear to be lymphoid or mast cells (Fig. 7). They possessed a somewhat hyperchromatic, convoluted nucleus, with a rim of well-stained and slightly granular cytoplasm, and the great majority did not stain metachromatically. We consider it probable that these cells are differentiating lipoblasts, as occasional 'transitional' cells with foamy vacuoles were also observed. At deeper levels, a variable number of small arteries and venules were seen, but they did not exhibit any particular abnormal features, and they were not accompanied by lipoblasts or sleeved with fat cells.

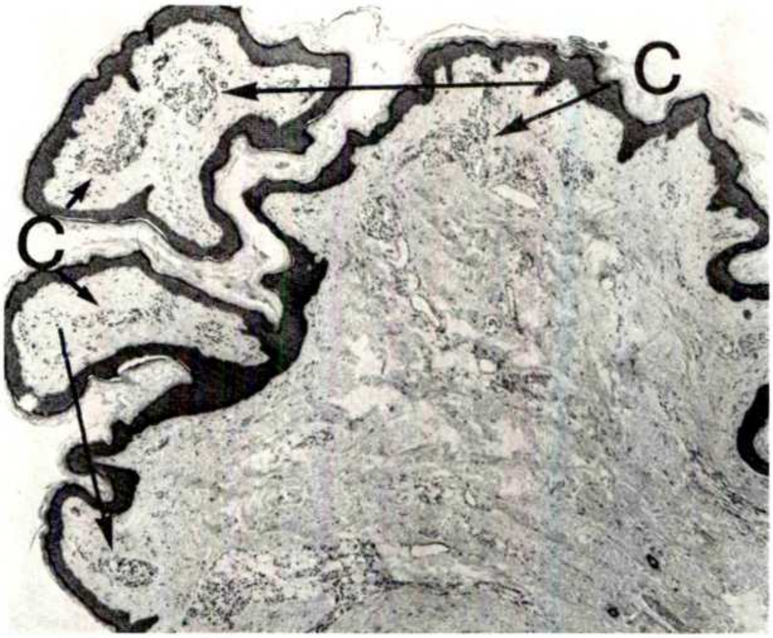


FIGURE 6. NSL. The 'angiomatous' stage is well shown. The vessels at the lower left side are surrounded by fat cells (see details Figs. 5 and 7) (H & E × 30). C, groups of tortuous capillaries.

The epidermis and appendages

In general, the epidermis and the appendages showed few distinctive changes. The epidermis over

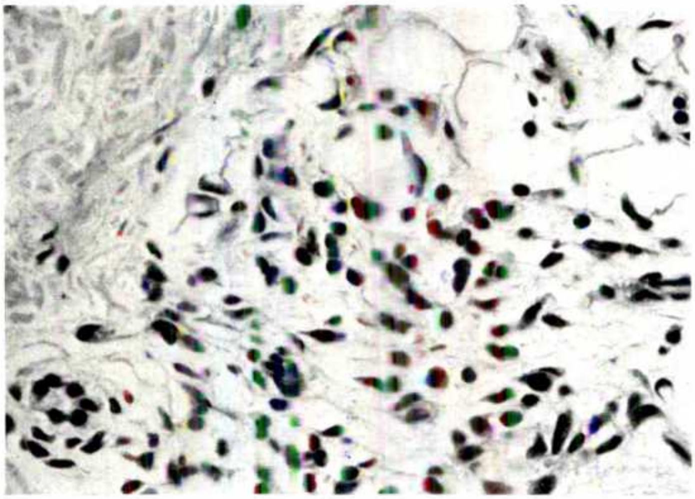


FIGURE 7. NSL. Detail from Fig. 6. Note the presence of mesenchymal cells surrounding the capillaries, including probable lipoblasts, pericytes and mast cells (H & E ×300).

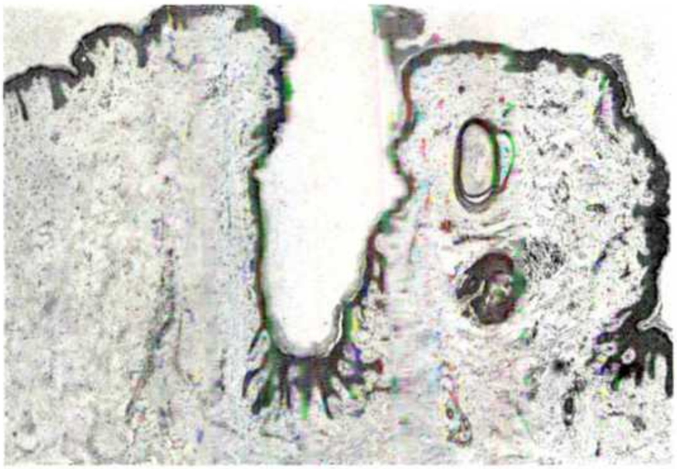


FIGURE 8. NSL. Irregular epithelial proliferation as seen at the base of the crypts. Small groups of fat cells are not apparent at this magnification. (From patient illustrated in Fig. 2) (H & E × 18).

the papules tended to run an undulating course similar to the normal skin of the flexures. Here and there elongations of the rete ridges were noted (Fig. 8). In some of the lesions deep crypt-like indentations were found, possibly corresponding to the comedo-like pores in which the lining epidermis showed considerable irregular downgrowth and branching of the rete ridges, suggesting a 'naevoid' abnormality (Fig. 8). In a few sections, dilated follicular canals were encountered, and occasionally groups of abortive hair-germ-like structures were seen, attached to the base of the crypts. However, no complex structures of the kind found in trichofolliculoma were found. In general both eccrine and pilosebaceous follicles were unaffected when encountered. It is possible that the numbers of these adnexal structures were reduced within the naevi as compared with the normal skin of the area.

Histochemistry and special stains

Delicate reticulin networks were found surrounding the superficial capillaries in areas with evidence of lipogenesis. In the same areas a network of PAS positive (not glycogen) staining material was also apparent around the vessels, insinuated between the perivascular, spindle and mononuclear cells already described. Metachromatic stains of unfixed cryostat sections also revealed some increase in metachromatic material in roughly the same distribution as the PAS positive material. Alkaline phosphatase activity (Azo dye coupling method) could be demonstrated in most of the capillaries in the superficial part of the naevi. The richness of the vascular supply was emphasized in 10 μm cryostat sections with standard adenosine triphosphatase technique (Wachstein and Miessel). No particular enhanced activity was seen in cells sleeving the superficial vessels, as might have been expected if these cells were differentiating into smooth muscle cells. Acid phosphatase activity, using an Azo dye technique, showed weak reaction only in and around the superficial capillaries, as compared with that of the granular cell layer of the epidermis.

DISCUSSION

The seven patients with multiple lesions correspond closely to the previously reported cases of NSL in the literature. The presence of many soft, skin-coloured to yellowish papules and plaques with a crinkled surface, covering a zonal area of the lower back, buttock or upper thigh is so highly characteristic that the condition should not be confused with other lesions. The plaques of juvenile elastoma may be yellowish in colour but are firm, and they present as a rubbery thickening of the skin, rather than as soft elevated excrescences. Juvenile elastoma tends to have a wider distribution over the trunk and limbs, although the lower back is a site of predilection. The histology is characteristic because of the increased amounts of thickened elastic fibres that encase the dermal collagen.

The lower back and trunk are also common sites for other connective tissue naevi, whether or not occurring as part of the tuberous sclerosis syndrome; this heterogeneous group of lesions is discussed in detail by Pierard & Kint (1966), and Rocha & Winkelmann (1962). The comonest type, the so-called 'cobblestone naevus', usually occurs in the lumbar region and may be bilateral. The lesions may not be apparent unless the skin be stretched or the patient observed in oblique light, and they are often more easily felt than seen, as a change in texture on palpation. They do not show ectopic fat or increased elastic tissue.

The status of the thirteen solitary lesions we report is more problematical. It could be argued that these lesions represent nothing more than giant skin tags showing fatty herniation. Indeed, as Weitzner (1968) has pointed out, the solitary type may occur at any site, and, as our data show, they tend to develop later in life. However, the solitary lesions do show a considerable predilection for the same sites as the rarer multiple type, and we believe that they represent a lesser form of NSL. It was not easy in every instance to make a fine distinction between a large skin tag and NSL on the basis of the histology, as some skin tags possess a large central core of fat with thinned dermis above, and in these instances fat can be present close to the surface. We have only included cases in our study in which isolated groups of fat cells were present around superficial vessels separated by dermis from the central fatty core.

The presence of plugged comedo-like lesions was a notable feature in two of our patients and, this has also been noted in patients reported by Lynch & Goltz (1958), Cramer (1960), Szodoray & Szondy (1961), Abel & Dougherty (1962) and Kurihara *et al.* (1967).

Our study strongly supports the view that NSL represents a naevoid abnormality in which the occurrence of ectopic fat in the upper levels of the dermis is but one facet. We have been unable to find any evidence to support the hypothesis that fatty metaplasia is secondary to degeneration of the collagen, as originally propounded by Hoffmann & Zurhelle (1921). We are impressed by the great variability in the amount of ectopic fat, and its virtual absence in some of the smaller papules when several papules were examined histologically from the same patient. It seems to us that lipogenesis in NSL appears to 'recapitulate' the way fat develops in the primitive mesenchyme of the fetus. In the fetus, the sequence of events is, first, development of a capillary network from a primordial vessel followed by the development of spindle and stellate mesenchymal cells around the vessels, some of which give rise to primitive lipoblasts. These cells possess a somewhat hyperchromatic nucleus and a rim of finely granular eosinophilic cytoplasm. In these cells small peripheral fat vacuoles develop which later coalesce. By the 12 mm embryo stage, separate but discrete lobules of recognizable fat are present (Fig. 9). At this stage, about 50% of the cells resemble small, adult lipocytes, and they contain a single vacuole that displaces the nucleus to the side of the cell. However, many undifferentiated mesenchymal cells and a rich capillary network are still present, particularly at the periphery of the developing fat lobule. The rare benign tumour of infancy, lipoblastomatosis, is composed of circumscribed lobules of 'primitive' fat tissue, separated by fibrous septa. The presence of 'myxoid' features and well-marked vascularity in lipoblastomatosis allow a differentiation from hibernoma (Vellios, Baez & Shumacker, 1958; Kauffman & Stout, 1959), an uncommon tumour resembling 'brown fat' tissue of the fetus. It is of interest that lipoblastomatosis shows no special tendency to occur around the pelvic girdle, and only four out of thirty-five lesions affected the buttock in the recent report of Chung & Enzinger (1973). Angiolipoma is another lipomatous tumour in which a rich capillary network is a characteristic feature, particularly at the periphery of the lesion. We interpret angiolipoma as the early angioblastic stage in the formation of a lipoma, rather than such vascularity being a secondary change in a mature lipoma, as suggested by Howard & Helwig (1960).

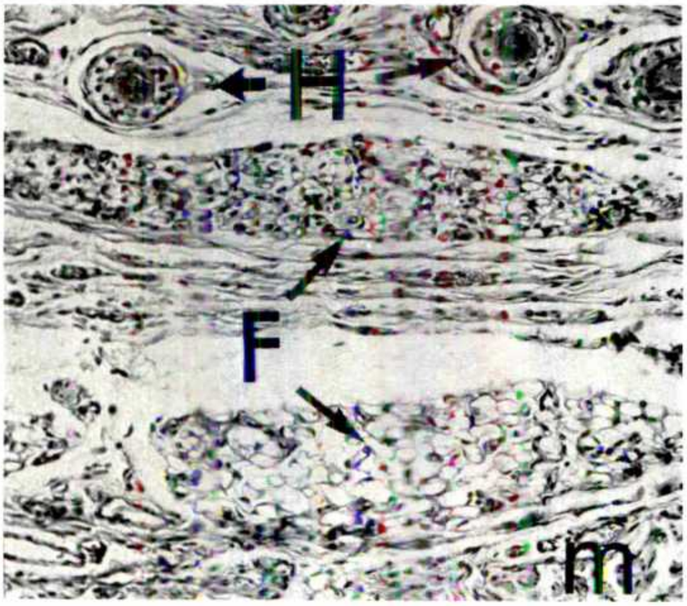


FIGURE 9. 13 mm human embryo chest wall below axilla. Ovoid lobules of primitive fat tissue are present. H, Hair follicles; M, Muscle; F, Fat lobules.

In NSL we have gained the impression that an inverse relationship exists between the vascularity and the presence of mature fat. It was also apparent that the proliferating superficial capillaries in NSL were accompanied by a prominent number of adventitial cells. Among these cells we were impressed by the presence of somewhat hyperchromatic mononuclear cells possessing a granular cytoplasm which did not stain metachromatically. We think it likely that most of these cells represent primitive lipoblasts, and we have been able to detect similar cells in the vascular parts of several angiolipomas. Increased connective tissue mucin is also a feature in those parts of NSL showing angioplasia, but we have not encountered areas with any striking myxoid changes of the order that is found in myxoid liposarcomas (Enterline *et al.*, 1960). Adult common moles (cellular naevi) sometimes contain groups of mature fat cells, particularly in older persons (Fig. 10). Occasionally this fatty infiltration can be so extensive that the presence of residual naevus cells may be overlooked, leading to an erroneous histological diagnosis of NSL. The stimulus to such fatty metaplasia in moles is unknown.

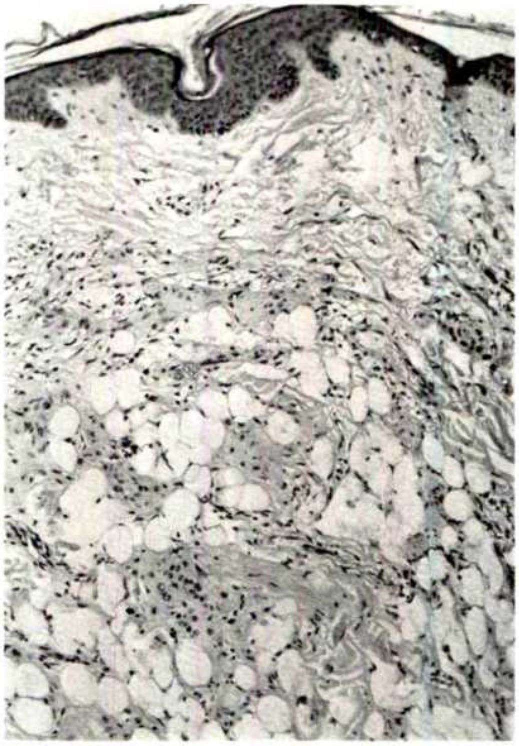


FIGURE 10. Cellular naevus of scalp of woman of 64 years showing marked fatty metaplasia. (H & E × 40).

We have no explanation as to why NSL shows such a marked predilection for the 'pelvic girdle' area. Diffuse accumulation of fat over the buttocks and thighs may occur paradoxically in some of the lipodystrophy syndromes. Finley & Musso (1972) pointed out that the buttock is a site of fat storage in Bushmen and Hottentots (steatopygy). However, to postulate a 'lipophilic' zone in this part of the body is perhaps too far-fetched an explanation to help account for the peculiar localization of NSL in man.

ACKNOWLEDGMENTS

R.Marks is in receipt of a grant from the M.R.C. We are grateful to the various clinicians at St John's who have allowed us to use their cases for this study. We acknowledge with gratitude technical help in the preparation of histology slides, Mr E.A.Wheeler; histochemical techniques, Mr D.Ramnarain; and photography, Mr S.Robertson.

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Reactions to aspirin and food additives in patients with chronic urticaria, including the physical urticarias*

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Accepted for publication 11 November 1974

SUMMARY

In 131 patients with chronic urticaria, including physical urticarias, oral provocation tests were done with aspirin. A total of thirty-one patients showed a reaction on aspirin challenge. Reactions were seen in 35% of patients with idiopathic urticaria, 52% of patients with cholinergic urticaria, and 43% of those with pressure urticaria.

The patients with reactions to aspirin were also tested with tartrazine, sodium benzoate, 4-hydroxy benzoic acid, sodium- and phenyl salicylate and the analgesics indomethacin, paracetamol and mefenamic acid. In nineteen of twenty three aspirin sensitive patients, positive reactions to one or more of these substances were observed. Indomethacin and tartrazine had the highest scores. There was no statistically significant correlation between aspirin reactions and the presence of nasal polyposis, sinusitis, asthma or atopy.

It has been shown that aspirin is an important eliciting factor in a considerable percentage of patients with chronic idiopathic urticaria and asthma (Moore Robinson & Warin, 1967; Champion *et al.*, 1969; Michaëlsson & Juhlin, 1973; Settipane & Chafee, 1974).

So far only one group of investigators has tested patients with physical urticarias for aspirin sensitivity, with negative results (Moore Robinson & Warin, 1967). In aspirin sensitive patients with chronic urticaria and asthma, reactions have also been described to certain food additives and analgesics (Michaëlsson & Juhlin, 1973; Smith, 1971). During an investigation of the pathogenesis of chronic urticaria, it was noticed that a history of aspirin sensitivity was present in some patients with physical urticarias, as well as in those with chronic idiopathic urticaria. Therefore it was decided to perform aspirin provocation tests in a group of patients with chronic urticaria, including many patients with physical urticarias. Patients with positive aspirin provocation tests were also tested with tartrazine, benzoates, salicylates and the analgesics indomethacin, paracetamol and mefenamic acid.

All urticaria patients were screened for the presence of nasal polyposis, sinusitis, asthma and atopy.

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* Read in part before the 9th European Congress of Allergology and Clinical Immunology, London, September 1974.

Patients

A group of 131 patients with urticaria of more than 3 months' duration observed over a 3-year period was tested. All had been studied intensively for the presence of physical urticarias. The criteria for diagnosis and the tests used have been described. (Doeglas & Bleumink, 1975.)

Included were patients with urticaria factitia, delayed pressure urticaria, cold urticaria, cholinergic urticaria, patients without physical urticaria (chronic idiopathic), and acquired angio-oedema (Table 1).

TABLE 1. Type of urticaria and results of provocation tests among patients with chronic urticaria

Type of urticaria	No. of patients	Percentage	Provocation test positive
Physical urticarias			
Urticaria factitia	22	16·8	3 (14%)
Pressure urticaria	16	12·2	7 (43%)
Cold urticaria	18	13·7	0
Cholinergic urticaria	17	12·9	9 (52%)
Heat contact urticaria	1	0·7	1
Total	74	56·4	20 (27%)
Chronic idiopathic urticaria	50	38·1	16 (32%)
Angio-oedema (acquired)	7	5·4	1 (14%)
Total	131	100·0	37 (26%)

Provocation tests were done during a phase of minimal or absent urticaria. Antihistamines and corticosteroids were discontinued for at least a week before the tests. Most patients were studied as out-patients.

Chemicals used for provocation tests

The dosages and structural formulae are shown in Table 2. They were administered in gelatin capsules with lactose added as filling.

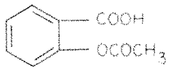
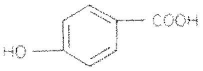
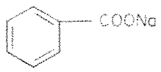
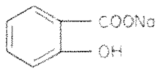
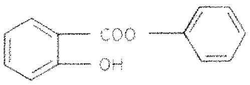
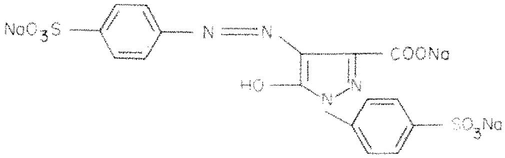
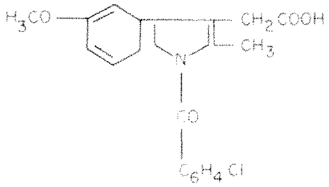
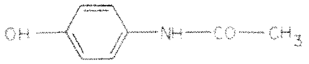
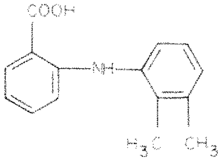
Test method

Urticarial lesions present before testing were marked with a skin pencil. Aspirin was given first. Patients with a history of aspirin sensitivity, asthma, nasal polyposis, recurring sinusitis or rhinitis were given the lowest dosage of aspirin (2 mg). The dosage was increased each hour until symptoms occurred. Patients with a negative history received 1000 mg at once. The patients were observed for 2 h and after 24 and 48 h. Reactions occurring within 2 h were observed until symptoms had subsided. Doubtful reactions were evaluated in comparison with the placebo or the test was repeated at a later date. After a positive reaction no further tests were done for a week. No more than one substance was tested on each day. Only those patients who showed a reaction to aspirin were tested with the other chemicals as indicated in Table 2.

Evaluation of reactions

A provocation test was considered positive if the patients developed urticaria or angio-oedema after it had been absent or if a dramatic increase occurred in the lesions already present, with or without

TABLE 2. Dosages and structure of chemicals used for provocation test in patients with chronic urticaria

Chemical	Dosage (mg)	Structural formula
Aspirin	2;5,10;50;100 250;500,1000	
4-hydroxybenzoic acid	50,100	
Sodium benzoate	250,500	
Sodium salicylate	500,1000	
Phenyl salicylate	500,1000	
Tartrazine	2,5;10	
Indomethacin	25,50	
Paracetamol	500	
Mefenamic acid	250	
Lactose placebo	300	

additional symptoms. Additional symptoms observed were: a scarlet flush beginning in the scalp and spreading over the face and chest, conjunctival injection, increased tear secretion, nasal congestion, watery rhinorrhoea, cough, hoarseness, wheezing and dyspnoea or asthma.

Severe reactions all occurred within 2 h, delayed symptoms might occur up to 24 h.

Nasal polyposis, recurring sinusitis

A careful search for these symptoms was made in the history and by examination in the E.N.T. department. Roentgenograms of the paranasal sinuses were made when there was the slightest suspicion of pathology present.

Presence or absence of atopy

Patients were classified as atopics if they had a positive reaction to one or more of 3 intracutaneous skin tests with house dust (0·1%), human dander (0·1%) or animal dander (0·01%) (Haarlem Allergen Laboratory, the Netherlands), together with evidence of asthma, seasonal hay fever or atopic dermatitis in the patient or his first degree relatives. Histamine (i.c.) 0·1, 0·01, 0·001 mg/ml was used as a standard for the skin tests. The results in the aspirin positive and negative urticaria groups were compared with those of a control group of skin disease patients from the same clinic without atopic dermatitis. The control group was matched for sex and age and was questioned and tested specifically for this purpose.

Diet free of tartrazine, benzoates and salicylates

In the Netherlands the use of food additives is regulated by law. All foods in which the law permitted the use of tartrazine or benzoates were forbidden. Fruits known to contain salicylates were also eliminated.

Determination of protease inhibitors and complement values in plasma

Determinations of anti-trypsin, anti-chymotrypsin and anti-kallikrein activity of plasma of aspirin sensitive patients and controls were performed according to methods described in earlier papers (Doeglas & Bleumink, 1974, 1975).

Measurements of levels of C1 esterase inhibitor, alpha$_1$-antitrypsin and alpha$_2$-macroglobulin and the complement factors C3 and C4 were described in the same papers. Micro haemolytic complement titration of plasma was performed after Levine (1967). The data are recorded as CH50 values or as the reciprocal of the amount in ml in which 50% of 5×10^7 sensitized sheep erythrocytes will lyse in 1 h incubation at 37°C.

RESULTS

The group of 131 patients contained 57% of patients with physical urticarias (Table 1). This high percentage probably reflects the type of urticaria patient referred to a University Clinic, but is also a result of an active search for physical causes (Doeglas & Bleumink, 1975).

In thirty-seven of the 131 patients (26%) a positive provocation test with aspirin was observed (Table 1). There were notable differences in the incidence of aspirin sensitivity in the subgroups. The highest incidence (52%) was in the patients with cholinergic urticaria, followed by 43% in the group with delayed pressure urticaria and 32% in the group with chronic idiopathic urticaria. Lower incidences were observed in the patients with urticaria factitia and acquired angio-oedema, while none of the patients with cold urticaria showed positive reactions.

One patient with contact heat urticaria had marked angio-oedema after aspirin administration. There were thirteen females and twenty-four males in the aspirin positive group and fifty-four females and forty males in the aspirin negative group. The sex distribution in the various types of urticaria was unremarkable.

The type of skin symptoms noticed in the provocation tests consisted of urticaria in thirty-six patients, angio-oedema in fifteen, and a very typical scarlet flush beginning in the scalp and spreading downward to the shoulders and chest in eight patients. In nine patients there were eye symptoms such as injection of the conjunctiva, increased tear secretion and burning; in seven patients nasal congestion, sneezing and a running nose were observed; four complained of an irritable cough, four had swelling of the throat or hoarseness and nine complained of various degrees of dyspnoea. In individual patients tachycardia, gastric hyperacidity and pain, fever and purpura occurred. These additional symptoms were seen predominantly in the patients with cholinergic and with chronic idiopathic urticaria.

There was evidence of previous aspirin reactions in the history of only ten patients, five had noted swelling of the eyelids or lips and two patients with cholinergic urticaria had suffered a severe reaction with systemic symptoms. Among these ten patients the reaction could be reproduced by provocation tests in seven. In thirty patients without a history of aspirin reactions, the test was also positive; nine of these patients claimed never to have used aspirin-containing drugs before.

The question of a quantitative effect of aspirin was studied in nine patients who received graduated doses. The lowest doses to which they responded were 50 mg in two, 100 mg in one, 250 mg in three, 500 mg in one and 1000 mg in two patients. In four of these patients the response was doubtful at these dosages, and they produced a more clearcut reaction on the next higher dose. In one patient 500 mg was given after a slight erythema of the scalp had been produced with 50 mg. He developed the most severe reaction which we have seen, with lacrimation, running nose, flush, urticaria, angio-

TABLE 3. Provocation tests with other chemicals in aspirin sensitive patients with chronic urticaria

Type of urticaria	Patient no.	Sex	Tart.	4-OH benz.	Sod. benz.	Sod. sali.	Phenyl sali.	Indo meth.	Para cet.	Mefen. acid
Urticaria factitia										
	1	F	–	–	–	–	+	–	–	–
Pressure urticaria										
	2	M	+	–	+	–	–	–	–	–
	3	F	–	–	–	+	+	–	–	–
	4	M	–	–	–	–	–	–	–	+
Cholinergic urticaria										
	5	M	+	–	–	–	–	+	–	–
	6	M	–	–	–	–	–	+	–	–
	7	F	–	–	+	o	o	–	–	–
	8	M	+	–	–	–	–	o	o	o
	9	F	–	–	–	–	–	–	–	–
Chronic idiopath. urticaria										
	10	F	+	+	+	+	–	+	–	+
	11	F	+	–	–	+	–	+	+	–
	12	F	–	+	+	–	–	+	–	–
	13	M	–	–	–	+	+	+	–	–
	14	F	+	+	–	o	o	+	–	–
	15	F	–	+	–	–	–	–	–	–
	16	M	–	–	+	–	–	–	–	–
	17	M	–	–	–	+	o	o	o	o
	18	M	–	–	–	–	+	–	–	–
	19	M	–	–	–	–	–	+	–	–
	20	F	–	–	–	–	–	–	–	–
	21	M	–	–	–	–	–	–	–	–
	22	M	–	–	o	o	o	o	o	o
Angio-oedema (acq.)										
	23	F	+	–	–	+	+	+	–	+
Positive/tested			7/23	4/23	5/22	6/20	5/19	9/20	1/20	3/20
Percentage			30·4	17·4	22·7	30·0	26·3	45·0	5·0	15·0

oedema and an asthma attack. After the dosages of 100 and 250 mg had been introduced, such severe reactions have not been seen.

The interval between ingestion and symptoms was variable. In thirty-two positive aspirin provocation tests the interval was less than 2 h; this included the only two severe reactions which were seen. In twenty-two tests it was less than 12 h and in two instances between 12 and 24 h.

The duration of the symptoms induced by the provocation tests was usually from a few to 24 h. In some patients exacerbations of the urticaria were induced lasting a week.

For reasons of expedience, tests with the other chemicals could be performed in only twenty-three of the thirty-seven aspirin sensitive patients (Table 3). There was no correlation between any particular type of urticaria and a reaction to a specific chemical.

The highest incidence of reactions was found to indomethacin, to which nine of twenty (45%) patients responded, whereas seven of twenty-three (30·4%) reacted to tartrazine, six of twenty (30%) to sodium salicylate, five of nineteen (26·3%) to phenyl salicylate, five of twenty-two (22·7%) to sodium benzoate, four of twenty-three (17·4%) to 4-hydroxybenzoic acid, three of twenty (15%) to mefenamic acid and one of twenty (5%) to paracetamol. Only one of the nine patients responding to indomethacin had ever used the drug before, only one of four had ever used paracetamol and none had used mefenamic acid.

The incidence of nasal polyposis and asthma (Table 4) did not differ significantly between the aspirin positive and negative groups. Sinusitis occurred more often in the aspirin positive group.

TABLE 4. Aspirin sensitivity in chronic urticaria patients related to nasal polyposis, sinusitis and asthma

	Aspirin provoc. negative 91	Aspirin provoc. positive 33	Total 124	Significance
polyposis	4 (4·4%)	3 (9·1%)	7 (5·6%)	n.s.
sinusitis	1 (1·1%)	3 (9·1%)	4 (3·2%)	$P = 0{\cdot}02\text{–}0{\cdot}05$
asthma	6 (6·6%)	1 (3·0%)	7 (5·6%)	n.s.

Among ninety-four patients with chronic urticaria and a negative aspirin provocation test, there were nineteen who could be classified as atopics (22·1%). In the aspirin positive group there were four of thirty-seven (10·8%) and in the control group three of fifty (6·0%).

The differences between the aspirin positive and negative groups are statistically not significant. Atopics did occur significantly more often in the aspirin negative group compared to the controls.

Patients sensitive to aspirin received a diet free from tartrazine, benzoates and salicylates. The diet was used by twenty-seven patients. The results were good to excellent in seventeen of them, during a follow-up period of 1–8 months.

Several patients noted exacerbations after the use of forbidden foods. It was also noticed that after the disappearance of the urticaria, some of the patients were able to resume a normal diet without recurrence of the symptoms.

In a previous study, a number of protease inhibitors and the complement values C3 and C4 were determined in the plasma of ninety-two patients with chronic urticaria and a control group of patients with other skin diseases. The same determinations were performed in the plasma of twenty-one patients with aspirin sensitive urticaria and compared with a control group of sixty-eight patients with other

TABLE 5. Protease inhibitors and complement factors in patients with aspirin hypersensitivity

	No.	C1 esterase inhibition	Trypsin inhibition	Chymotrypsin inhibition	Kallikrein inhibition	Alpha 1-anti-trypsin	Alpha 2-macro-globulin
Patients with aspirin hyper-sensitivity	21	0·99±0·32	1·369±0·281	0·787±0·271	0·146±0·044	313±93 (n=20)	222±73 (n=20)
Controls	68	0·86±0·28	1·251±0·266	0·701±0·332	0·139±0·033	273±72	268±83

(No significant differences, Student's *t*-test.)

skin diseases. There were no significant differences for the levels of protease inhibitors (Table 5), and in particular the values for C3, C4 and total haemolytic complement (Table 6), were normal.

In seventeen plasmas collected during the acute phase of aspirin reactions after aspirin provocation tests, the values for C3, C4 and total haemolytic complement did not show deviations from the control group (Table 6).

TABLE 6. Complement factors C3 and C4 and total haemolytic complement in aspirin sensitive patients and controls

	No.	C3	C4	Haemolytic complement*
Patients in quiescent phase	21	87±18	37·6±10·1	Not done
Patients in active phase after provocation	17	81±10	29·2±7·4	116±31 (n=12)
Controls	68	80±14	32·5±10·7	98±23 (n=34)

* Micro complement titration: CH50 values of 5×10^7 cells.

DISCUSSION

The reported incidence of aspirin sensitivity in chronic idiopathic urticaria is between 21 and 75% (Moore Robinson & Warin, 1967; Champion *et al.*, 1969; Michaëlsson & Juhlin, 1973), and between 2 and 10% in asthma (Storm van Leeuwen, 1928; Settipane & Chafee, 1974). Provocation tests with aspirin, food additives and analgesics have been carried out in urticaria patients and in asthmatics (Smith, 1971, McDonald, Mathison & Stevenson, 1972; Michaëlsson & Juhlin, 1973). These tests should be carried out only under strict supervision in a hospital environment. In our patients with chronic idiopathic urticaria the incidence of aspirin sensitivity was 32%, which agrees well with the published findings. Our study included a large number of patients with physical urticarias and an unexpected finding was the high incidence of aspirin sensitivity among patients with cholinergic urticaria and pressure urticaria. Moore Robinson & Warin (1967) studied the effect of 300–1200 mg of aspirin on the outcome of tests for physical urticaria in ten patients with dermographism, three with cold urticaria and five with cholinergic urticaria and found no difference in the reaction to the test before or after aspirin. Moreover, there was no urticarial eruption reported after the use of aspirin alone. The small number of cases with cholinergic urticaria in their series may have obscured the aspirin sensitivity. In the case of pressure urticaria four of the seven aspirin responsive patients also had spontaneous weals, which may have influenced the results. Michaëlsson & Juhlin (1973) excluded patients with physical urticarias from their study.

The quantitative effect of aspirin in aspirin-sensitive patients noticed by Moore Robinson & Warin was confirmed in four of our patients who received graded dosages.

The incidence of sensitivity to tartrazine and benzoates was lower in this study than in that of Michaëlsson & Juhlin (1973). This may be explained in part by our use of aspirin as a screening agent, whereas sensitivity to tartrazine and benzoates may occur without aspirin sensitivity.

The finding that only one of nine indomethacin sensitive patients, one of four paracetamol sensitive and none of two mefenamic acid sensitive patients had ever used the drug before, makes a previous allergic sensitization questionable. Samter & Beers (1968) also reported indomethacin sensitivity in aspirin sensitive asthmatics who had never used the drug. Smith (1971) described sensitivity to paracetamol and mefenamic acid in aspirin sensitive asthmatics. Reports on sensitization to sodium- and phenylsalicylate are conflicting. Our results show that at least some aspirin sensitive patients do not tolerate them, confirming the findings of Moore Robinson & Warin (1967) who noticed worsening of urticaria in thirteen of eighteen aspirin sensitive patients with sodium salicylate and in two of three after phenyl salicylate. Samter saw no reaction after sodium salicylate in forty aspirin sensitive asthmatics, whereas Storm van Leeuwen (1928) and James (197[illegible]) noticed reactions with sodium salicylate in aspirin sensitive asthmatics, although relatively higher dosages were needed. The role of salicylates in foods is probably limited since they are no longer used as additives in most countries and they occur only in small quantities in natural foods (Juhlin & Michaëlsson, 1973).

Aspirin sensitivity in asthmatics occurs especially in those who have nasal polyposis and sinusitis (Giraldo, Blumenthal & Spink, 1969; McDonald *et al.*, 1972; Falliers, 1973). This combination has been called the asthma triad (Samter, 1968). An incidence of 48–73% of polyposis and sinusitis in aspirin sensitive asthmatics has been reported (Giraldo *et al.*, 1969; McDonald *et al.*, 1972; Settipane & Chafee, 1974).

The search for polyposis and asthma in our urticaria patients did not reveal a significant correlation with aspirin sensitivity. Only one patient had the 'asthma triad' as well as his urticaria. Sinusitis was slightly more frequent in the patients with aspirin reactions, but the evidence is based on a rather small number of cases.

Aspirin sensitivity in asthma patients is found mostly in those with the intrinsic non-atopic type of asthma with predominantly negative skin tests to inhalants (Samter, 1968; Falliers, 1973; Chafee & Settipane, 1974). There was no evidence of a decreased number of atopics in our aspirin sensitive urticaria patients compared with the other urticaria patients or the controls. Apparently aspirin sensitive urticaria patients and asthmatics differ in the incidence of polyposis and sinusitis as well as in their relation to atopy.

Little can be said about the mechanism of action of aspirin in aspirin sensitive urticaria. Many studies that tried to implicate allergic mechanisms were largely unsuccessful (Giraldo *et al.*, 1969; Yurchak, Wicher & Arbesman, 1970; de Weck, 1971). The quantitative effect of aspirin, the cross reactivity with chemically non-related drugs, the fact that previous sensitization can usually be excluded and the disappearance of the aspirin sensitivity after regression of the urticaria, are inconsistent with an allergic sensitization. The only finding which aspirin, indomethacin, paracetamol and sodium salicylate have in common is their inhibitory effect on the synthesis of prostaglandins

PGE_1 and $PGF_{2\alpha}$ (Collier, 1971). These two mediators have opposed effects on vascular permeability and bronchial muscle (Willoughby, 1968; Collier, 1971).

Further study of prostaglandin metabolism might lead to a better insight into the pathophysiology of aspirin induced urticaria.

The finding of a high incidence of aspirin sensitivity in cholinergic, pressure and chronic idiopathic urticaria suggests perhaps a common denominator in the aetiology of these diseases.

Yurchak *et al.* (1970) suggested that a study of complement levels during acute reactions to aspirin seemed worthwhile. We have determined the plasma levels of C3 and C4 and total complement of twenty-one aspirin sensitive patients in a quiescent phase and seventeen plasma samples obtained during the acute phase of aspirin reactions, after aspirin provocation tests. There were no deviations from the values of a control group. This suggests that major changes in the complement system are not present during the acute phase of aspirin reactions. Definite proof can be given only when all the individual components of complement are measured.

ACKNOWLEDGMENTS

I am grateful to Ir. H. Cats, Keuringsdienst voor Waren, Rotterdam for his help in preparing a diet free from the incriminated food additives. I wish to thank the staff of the E.N.T. Department (Chief Professor Dr P.E.Hoeksema) for the examination of the urticaria patients. Dr E.Bleumink, biochemist, Department of Dermatology, University Hospital, Groningen performed the enzyme studies.

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British Journal of Dermatology (1975) **93**, 145.

One hundred twin pairs patch tested with primary irritants*

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Accepted for publication 29 November 1974

SUMMARY

The cutaneous sensitivity to benzalkonium chloride, sodium lauryl sulphate and potash soap was determined in 54 monozygotic and 46 dizygotic twin pairs. Comparing the intra-pair reaction strength a higher degree of concordance was found among monozygotic than among dizygotic twins, and even more so when monozygotic twins were compared with matched controls.

Irritant dermatitis, or 'traumiterative' eczema, constitutes a major problem among hand eczemas. Research on irritant dermatitis has often been based on the assumption that it might be aetiologically connected with an increased sensitivity to primary irritants. For any individual patient such hypersensitivity is, however, usually specific to one or more substances and the skin may react weakly to other compounds (Björnberg, 1968). What governs the cutaneous sensitivity to primary irritants is essentially unknown. Since a constitutional background cannot be excluded a twin study was designed to investigate a possible genetic influence on the cutaneous sensitivity to three different irritants.

MATERIALS AND METHODS

From the Swedish Twin Register the addresses were obtained of same-sexed twins between the ages of 16 and 29 years living in the Malmöhus county. The twins were invited to a clinical and laboratory examination; 100 twin pairs volunteered and were paid for the study.

The *zygosity* of the twins was determined as follows:

(1) The twins were individually asked the question: 'As children were you as like as two peas so that mistakes of identity often occurred?'

(2) With both twins present, the hair colour, iris colour, and iris structure were compared.

(3) With doubtful results from the above, blood grouping was performed at the Blood Bank, University Hospital, Lund (Head: Dr B.Löw). This was found necessary in only seven twin pairs.

(4) Finger prints were secured and filed.

Patch tests were applied on the outer aspect of the right upper arm for 48 h and the reactions were read after a further 24 h. Al-test units (Imeco, Astra Agency Co., Sweden) were used, and fastened with Leukoplast (Beiersdorf, West Germany). Test substances, concentrations and vehicles were selected on the basis of Björnberg's investigation (1968) and of our own pilot studies. It was our purpose to elicit test reactions that were reasonably strong and individually varying. Thus, the following three test solutions were chosen: benzalkonium chloride (BC) 0·5% in water, sodium lauryl sulphate (SLS) 0·5% in water, and sapo kalinus (SK) (Potash soap) 20% in petrolatum.

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* Presented to the International Symposium on Contact Dermatitis, Gentofte, Denmark, 26 October 1974.

The *test reactions* were read without knowing the result of the zygosity determination. The reactions were graded along an arbitrary scale according to Björnberg (1968), but slightly modified. The following criteria were used for all substances:

0, no reaction; 1, 'effet de savon' (a slight non-inflammatory change in surface structure); 2, erythema, annular or patchy; 3, erythema, homogeneous; 4, erythema with questionable infiltration; 5, erythema with definite infiltration; 6, infiltrated erythema with vesicles, pustules and/or erosion.

For the *statistical analysis* the reaction to each substance in one subject was compared to that in another 'paired' subject and the difference in reactivity was recorded. The comparisons were made within three groups of pairs: monozygotic twins (MZ); dizygotic twins (DZ); and control pairs (C.) The control pairs consisted of randomly selected subjects from the entire material being non-related but of the same age and sex. The χ^2 test was used throughout.

RESULTS

The determination of zygosity among the 100 twin pairs showed the distribution of 54 MZ and 46 DZ.

The distribution of test reactions to the three primary irritants is given in Table 1. The majority of the reactions were fairly weak. On the basis of our arbitrary scale of 0–6, BC tested in the 200 subjects showed a median value of 3, SLS a median value of 1, and SK a median value of 1.

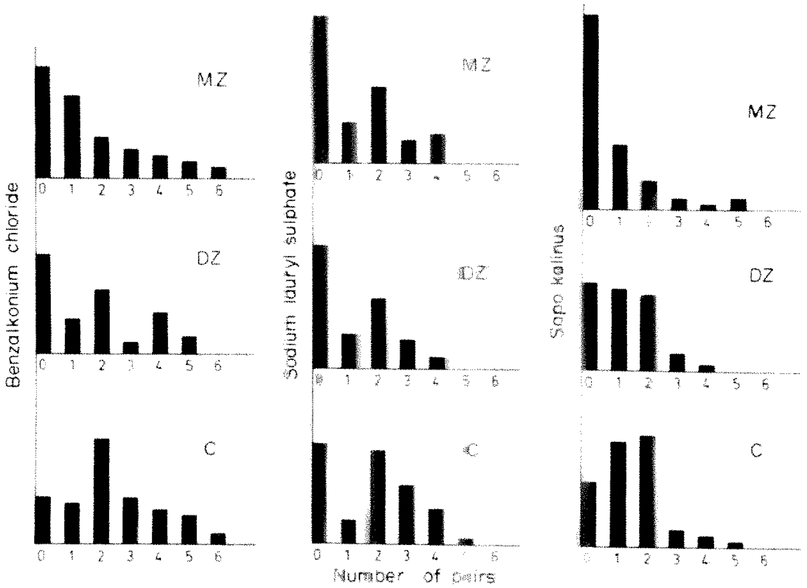

FIGURE 1. Test reactions to three irritants in three test groups. Ordinate: numbers of pairs; abscissa: intra-pair *difference* in test reaction.

TABLE 1. Distribution of test reactions to three primary irritants

Scale units	0	1	2	3	4	5	6
BC	57	7	35	27	36	17	21
SLS	98	3	50	25	19	4	1
SK	24	81	41	32	15	4	3

TABLE 2. The results of patch testing with three primary irritants in three test groups. For each substance the difference in reaction is based on an arbitrary scale

Difference in reaction	Amount of MZ pairs	Amount of DZ pairs	Amount of C pairs
		Benzalkonium chloride	
0	19	17	8
1	14	6	7
2	7	11	18
3	5	2	8
4	4	7	6
5	3	3	5
6	2		2
Totals	54	46	54
		Sodium lauryl sulphate	
0	25	21	17
1	7	6	4
2	13	12	16
3	4	5	10
4	5	2	6
5			1
6			
Totals	54	46	54
		Sapo kalinus	
0	33	15	11
1	11	14	18
2	5	13	19
3	2	3	3
4	1	1	2
5	2		1
6			
Totals	54	46	54

The result of patch testing with the primary irritants is given in detail in Table 2 and illustrated in Fig. 1. As may be seen there is a tendency to a greater intra-pair likeness in the reactions to each substance among MZ than among DZ, and even more so among MZ than among C.

The result of the statistical analysis is given in Table 3. For BC, a higher concordance was obtained among MZ than among C (significant). For SLS, there were no significant differences in concordance. For SK, a higher concordance was obtained among MZ than among DZ (significant), and among MZ than among C (highly significant). The analysis of the total material (mean of nine comparisons performed) showed the intra-pair concordance in test reactions to the three irritants to be MZ > DZ > C ($P > 0{\cdot}05$).

TABLE 3. Statistical analysis of concordance in test reactions: three comparisons made for each irritant. n.s., no significant difference

	MZ/DZ	DZ/C	MZ/C
Benzalkonium chloride	n.s.	n.s.	$P < 0{\cdot}025$
Sodium lauryl sulphate	n.s.	n.s.	n.s.
Sapo kalinus	$P < 0{\cdot}025$	n.s.	$P < 0{\cdot}0005$

DISCUSSION

The twin method for determining the genetic background to different biological parameters is now widely accepted. It is important, however, to rely on safe methods for zygosity determination. Among anthropological traits the intra-pair comparison of iris colour and structure as well as hair colour have been shown to be reliable (Essen-Möller, 1941). The diagnosis of MZ can, however, be made with a 99% safety from a questionnaire on the similarities between the twins as children (Cederlöf *et al.*, 1961). In the present work these two methods were combined and, furthermore, in a few doubtful cases blood grouping was carried out.

After patch testing with primary irritants a few subjects may demonstrate very strong reactions. To avoid this in the present material of healthy volunteers, test concentrations were chosen that eventually were found to be fairly weak. This was particularly true for SLS to which half of the subjects gave no reaction. This may partly explain why no significant differences were obtained with this compound. Also, the high frequency of 'effet de savon' type reaction to SK is apparently characteristic of that substance and not directly comparable with the low figures for the same reaction type using BC and SLS (Björnberg, 1968). On the other hand, the whole spectrum of reaction types was obtained with all three tests substances and 10% of the BC reactions were registered as being very strong.

There are certain factors known to influence the sensitivity of the skin to primary irritants. Marcussen (1963) has shown that during the first years of life there may be irritant reactions to ordinary patch tests with nickel and formaldehyde. In the age group tested in the present work no untoward sensitivity to primary irritants is known.

An atopic constitution may imply an abnormal reaction pattern to primary irritants, the sensitivity usually being increased (Rajka, 1967). Furthermore, a widespread or even limited eczema of any kind is accompanied by an increased reactivity to such compounds (Björnberg, 1968). In the present material a limited, non-classified eczema was observed in four MZ and five DZ subjects, in each pair one twin only being affected. The possible presence of an atopic constitution in the 200 subjects could not be explored on practical grounds. Even systemic disease is known to influence the reactivity to primary irritants. Thus it has been found to be lowered in patients with advanced cancer (Johnson, Maibach & Salmon, 1971; Mouridsen, 1974) and with lepromatous leprosy (Meneghini *et al.*, 1974), diseases obviously not relevant in the present material.

The twin method was used by Forsbeck, Skog & Ytterborn (1968) to elucidate the genetic background to delayed type allergies. No difference between MZ and DZ twins was demonstrated with respect to contact allergy, to tuberculin allergy, or to DNCB sensitization. These experimental findings agree with clinical records on allergic contact dermatitis among twins (Niermann, 1964). The tendency in our material to greater concordance among MZ than among DZ and C with regard to irritant reactions is in favour of a genetic influence.

ACKNOWLEDGMENTS

We are grateful to Associate Professors L. Kaij, Malmö, and R. Cederlöf, Stockholm, for making the Swedish Twin Register available, and to B. Ringnér, Dr.Sc., for performing the statistical analysis. The study was carried out with support from the Edvard Welander Foundation.

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British Journal of Dermatology (1975) **93**, 151.

Hypersensitive and insensitive extremities*

THE DERMATOLOGICAL ASPECTS

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Accepted for publication 15 November 1974

SUMMARY

The importance of physically intact hands and feet is taken for granted and consequently not appreciated until they are functionally impaired by vascular or neural injury or disease. The pathogenesis and clinical manifestations of cutaneous lesions complicating hypersensitive and insensitive limbs are described. The current therapeutic approaches and measures for these conditions are presented. Protection and prophylaxis against precipitating trauma for neurologically impaired parts are emphasized.

The function and importance of hands and feet are usually taken for granted until impaired by disease. Disease and injury to the neural or vascular system of the extremities are the two major sources of disability. When the peripheral nerves are injured and the autonomic fibres are damaged, not only sensory or motor changes may be appreciated but also vasomotor and sweating (autonomic) abnormalities occur. Such autonomic changes can occur with injury to peripheral nerves and also with peripheral or central injury to sympathetic fibres only, resulting in vasomotor, trophic, and sensory disturbances. Unlike the lesions of peripheral nerves, which may exhibit a conterminous absence of sensation and sweating, lesions of the nerve root exhibit sensory loss but preservation of sweating; spinal lesions affect sympathetic outflow so that there is a differential distribution between sympathetic and somatic sensory loss. The sudomotor fibres originating from the paraspinal sympathetic ganglia are distributed with the cutaneous blood vessels and innervate the sweat glands. Injury to these fibres usually results in the inability to sweat in the denervated area. Loss of sweating can be pinpoint, indicating an incomplete lesion or an incipient recovery, or diffuse. The denervated area may immediately become warm and red because of the paralysis of the sympathetic vasoconstrictor fibres. After this initial vascular vasodilator response, the receptor sites in the blood vessel wall become hypersensitive in a few weeks to circulating adrenaline and noradrenaline and cause a marked

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* Presented at the Symposium on the Management of Hypersensitive and Insensitive Extremities under the auspices and direction of Paul W.Brand, C.B.E., F.R.C.S., F.A.C.S., U.S. Public Health Service Hospital, Carville, Louisiana, 6–7 February 1974.

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and constant vasoconstriction. Because of the decreased blood supply, hands and feet become cool and dusky.

Hypersensitive and insensitive extremities precipitated by neural damage from occupation, injury, surgical operation, or disease may be accompanied by reflex neurovascular syndromes (Abbott & Mitts, 1974). The hypersensitive state can also develop in patients who have chronic chilblains or trench and immersion foot. The associated vasomotor and sweating changes may predispose or condition the patient to various complicating disorders. Two reflex sympathetic states may occur: causalgia, a relatively minor condition, characterized by hyperalgesia (burning pain), coldness, and hyperhidrosis of the affected area which is rarely red, warm and dry; and Sudeck's atrophy, a progressive and relatively rare major condition, which is usually post-traumatic and consists of severe causalgia, atrophy of tissue including muscle and bone, hyperkeratosis, onychodystrophy, and oedema of the affected part.

Among the clinical problems associated with the hypersensitive state are hyperhidrosis with or without bromhidrosis and keratoderma (the symmetrical lividity syndrome), and dyshidrotic eczema, which is seen not infrequently in patients with atopy. The atopic skin is more prone to eczematization when exposed to friction or maceration of wet feet. The constant pedal moisture invites epidermophytosis and allergic contact dermatitis, which may result from the leaching of materials from shoes and socks or from the local medicaments used to combat the hyperhidrosis or its undesirable side effects.

Treatment of the hypersensitive area depends on the nature of the problem, the degree of pain, and the response to therapy. The neural feedback mechanisms are distorted in both the hypersensitive and insensitive states. In the former, there is established a 'false image' and a concentration of the whole being in the pain area (causalgia). Consequently, the therapeutic approach is opposite to that of the insensitive state wherein physical stimuli, especially those potentially damaging, are avoided. In the hypersensitive condition, an increased input of touch sensation is advocated and is carried out by such means as walking barefoot on grass or sandy beaches, and the brushing of the involved site frequently and systematically with a nylon hairbrush. This state is usually accompanied by emotional instability. The judicious use of analgesics and tranquillizers, sympatholytic drugs, and, if necessary, sympathectomy is advocated.

The insensitive limbs result from a peripheral neuropathy caused by trauma, poisons (including drugs), alcoholism, nutritional deficiency, infections, metabolic disease, vasculitis, hereditary conditions, and congenital indifference to pain (Abbott & Mitts, 1974).

One of the major secondary cutaneous changes in the insensitive area is dryness, which not only reduces dexterity in the handling of small objects but promotes cracking, making the underlying tissues more vulnerable to such infections as cellulitis, synovitis, arthritis, and osteomyelitis (Fig. 1). When the hands are used without feedback of sensory information (insensitivity), they are more prone to being burned, cut, lacerated, or bruised (Brand, 1966). Normally, the pain of the injured digits reflexly inhibits their movement; however, since the insensitive hand does not have such a protective mechanism, the patient can further aggravate physically an already damaged part. Repeated physical insults and infections result in scarring, loss of blood supply and of tissue resiliency, and later in absorption of tissue and digits.

The skin of the foot, like that of the hand, is tough and has a tremendous capacity for responding to stress by becoming thicker. Although the subsequent pathological phenomena and principles are best seen and applied to the skin of the feet, other cutaneous sites may be similarly involved.

The main function of the sole of the foot is to spread diffusely the stress of weight bearing over the foot (Brand, 1966). The fat of the foot, unlike that of many parts of the body, cannot be moved about. The sole of the foot, through its firm, elastic, and compressible nature, spreads spatially and tem-

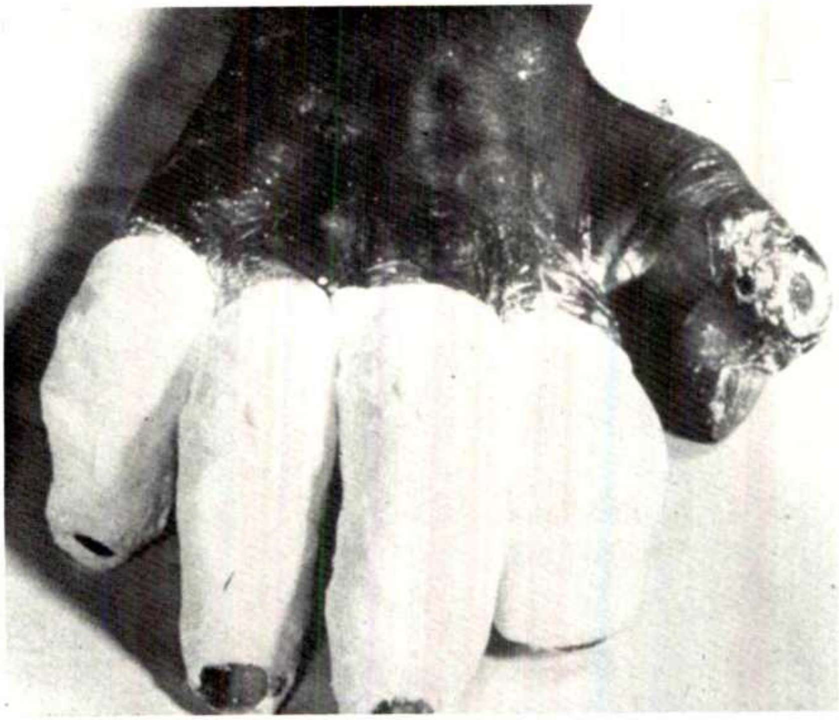

FIGURE 1. Recommended treatment for the fissured hands of the insensitive, the plaster casting of fingers in physiological position.

porally the strain of weight bearing and, by this action, preserves the skin. Like the insensitive area of the palm, the skin of the foot becomes dry, scaly, and even cracked. The flexural areas of the digits and the pressure areas of the palms and soles may develop superficial or deep fissures, which are potential niduses for secondary infections and hyperkeratosis.

Trophic ulceration of the skin of an insensitive limb, particularly the foot, is a serious complication. The basic cause of trophic ulceration is loss of sensation which denies the individual the ability to protect himself (Fig. 2); however, some still mistakenly harbour the idea that the ulceration results from the failure of the damaged nerve to supply some kind of nourishment to the skin. The basic mechanisms considered responsible for trophic ulceration are continuous pressure on, penetration into, and thrust of the involved skin (Brand, 1966).

When continuous pressure is sufficiently intense and sustained on a sensitive or insensitive area, the underlying tissue will be deprived of its blood supply and experience blister formation and ischaemic necrosis (Arndt, Mihm & Parrish, 1973). These changes are seen in paraplegics, comatose persons, and in patients who have improperly applied plaster casts. Although this mechanism is operative in the insensitive limb through the patient's failure, because of insensitivity, to make the necessary physical adjustments and accommodations, such as change of position to reduce sustained pressure to 'hot' points of feet, ulceration can result from a failure to remove injurious foreign bodies in the shoe. The common sites of trophic ulceration of the feet are in the area of the heel and beneath the metatarsal heads (Fig. 3). Foreign bodies may penetrate the insensitive skin (Fig. 4) and promote the formation of an abscess which, when drained spontaneously or surgically, results in painless ulceration of the skin.

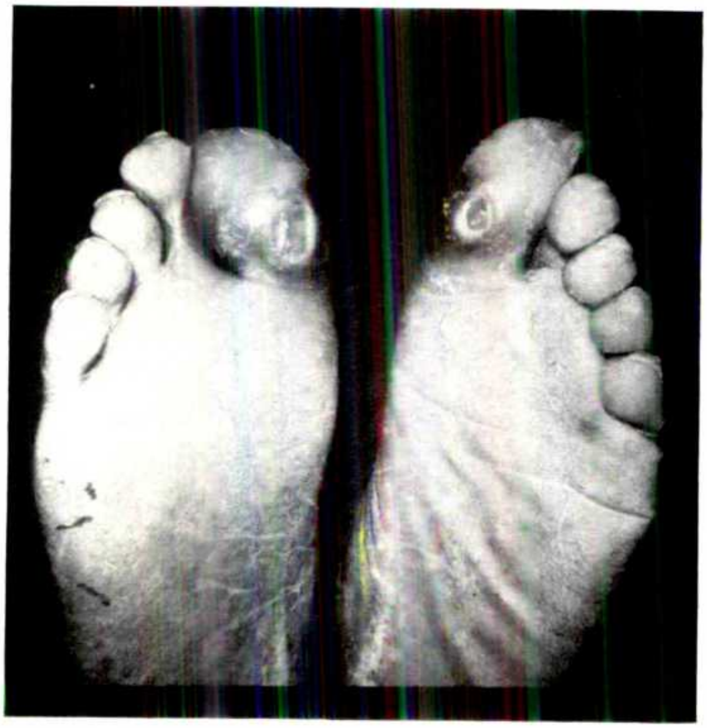

FIGURE 2. Trophic ulcers in a patient with insensitive feet resulting from alcoholic neuropathy.

The most common cause of trophic ulceration of the insensitive skin is the blunt injury resulting from 'thrust' or 'shear.' The overlying skin is more tough and resistant to injury than the underlying soft tissue between the skin and bone. The blunt injury traumatizes this area and produces bruising and haematoma. Patients with insensitive feet are susceptible to bruising because of the additive effect of the continuous trauma and the resultant secondary scarring and deformity. The insensitive area will continue to accept localized thrust or pressure; consequently, the patient with an insensitive foot continues to traumatize the bruised area, which is now the most prominent part of his foot and upon which the body weight is being concentrated. There is initially focal oedema and haemorrhage of the involved site and, later, necrosis with or without secondary infection and ulceration. The normal resiliency (visco-elastic properties) of the involved site is lost due to the resultant oedema and scarring which impede its natural inherent ability to absorb the mechanical trauma of shear (the sliding friction or horizontal stress).

When the foot has been ulcerated several times, a subsequent ulcer can penetrate to the bone and cause osteomyelitis and septic arthritis which will destroy the underlying bones, usually the metatarsal heads, and commonly result in a severe shortening of the metatarsal bones. The deformed foot, because of its reduction in size, is subjected to more weight per area. The clinical problem can be aggravated further by the downward projection of an affected and dislocated metatarsal bone into the ulcer, which is supporting more than its share of the body weight.

The shear force produces a more superficial lesion caused not only by the mechanical fatigue phenomenon (Comaish, 1973) but also by the accompanying local heat created by the sliding friction of the improper movement of the insensitive skin over another surface, such as a tight, ill-fitting shoe.

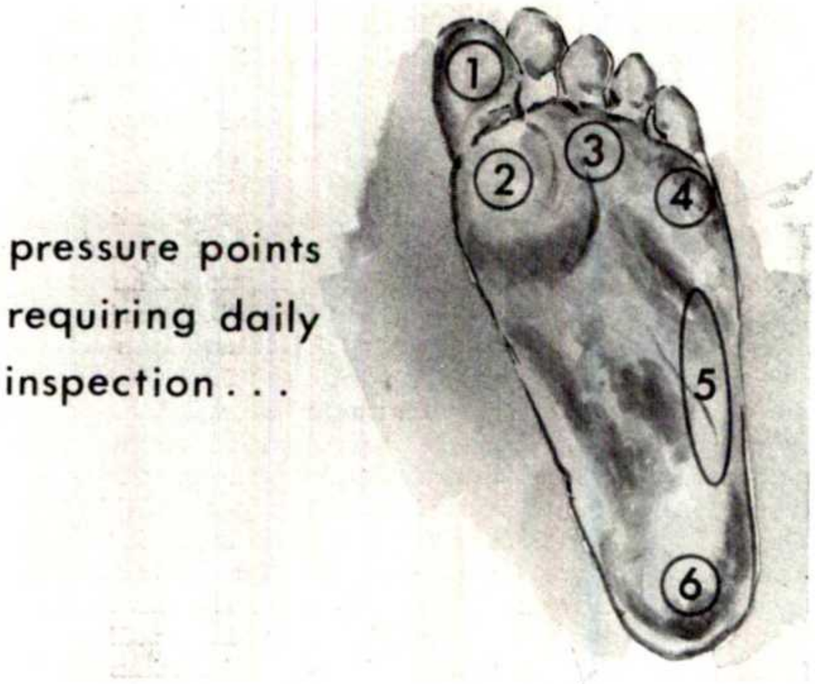

FIGURE 3. Common sites of trophic ulceration of the feet, the pressure points.

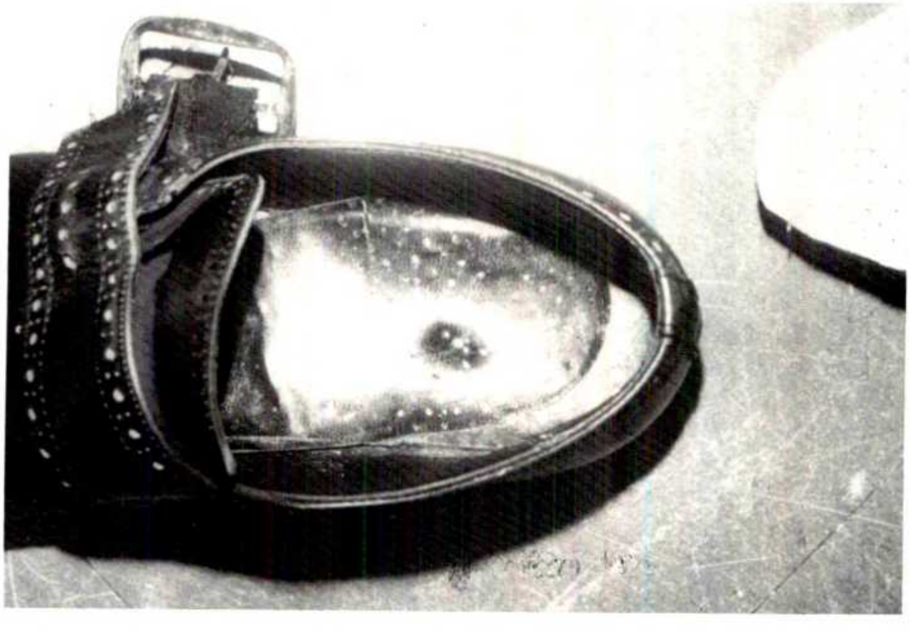

FIGURE 4. Cause of ulcer of right heel was a penetrating nail discovered only when patient removed inner sole of shoe.

Unfortunately, when motor paralyses accompany insensitivity of the hand or foot, as in leprosy, the anatomy of the involved areas is distorted by the resultant deformities, for example, the destruction of the ulnar nerve with development of the clawhand, of the median nerve with that of thenar atrophy, of the peroneal nerve with that of drop foot, and of the posterior tibial nerve with that of clawing of toes (Sabin, 1969). These changes increase the affected area's predisposition to pathological changes resulting from the forces of thrust, shear and pressure.

The approach to treatment of the insensitive extremities is to explain and to educate the patient in the inherent problems so that he can undertake the necessary prophylactic precautions and accept the medical, and when necessary, surgical procedures (Abbott & Mitts, 1974; Brand, 1966; Sabin, 1969; Wood, 1969). Patients with insensitive limbs must routinely inspect their hands and feet for patches of erythema, blisters, fissures, haematomas, presence of foreign bodies and ulcers. Areas of deep tenderness should be searched for routinely by pressing on the sites of hands and feet exposed to the greatest pressure. Patients should watch for darkening of scars as this could be caused by haemorrhage. Scars and calluses should not be allowed to become too cornified or thickened because they can act as 'lumps' in the shoe. Patients are instructed and encouraged to substitute visual control for the absent cutaneous sensation and to develop a new warning system by rerouting sensory information to the central nervous system through the remaining intact sensory systems of vision and hearing. Such visual control is effective for the avoidance of the more obvious hazards in the environment except those resulting from the fine, dexterous movements of the finger. The latent dangers to those susceptible digits are the use of exaggerated forces on unpadded scissors, hand tools, and small knobs or pot lids and the careless and inconsiderate grasping of metal handles of heated utensils and uninsulated coffee cups which conduct heat and burn insensitive skin. Patients should be aware of the dangers in such ordinary activities as smoking, or, especially in leprosy, turning a key. Protective gloves should be worn. Vinyl impregnated cloth gloves are useful because they conform well to the fingers, provide friction to facilitate grasping, and resist dust. When the insensitive digit is injured, the 'natural' splint, pain, is absent and should be replaced by an external splint, which is prophylactically essential to abort further damage.

Dryness of the skin is best handled by frequent and prolonged soaking in cool water followed by the immediate application of vaseline. When excessive callus formation is present, not only mechanical debridement (paring) but also the application of a keratolytic preparation such as Keralyt (a preparation containing salicylic acid, propylene glycol and water—Westwood Pharmaceutical Co.) should be used with or without plastic occlusion; care must be taken to avoid the potential irritation by keratolytics. Fissured areas may be similarly treated with the addition of a fluorinated corticosteroid ointment with or without occlusion or vaseline. Any clinical evidence of secondary infection should be treated by topical and, when necessary, systemic antibiotics. Any patient with a localized infection should be put to bed and treated with the appropriate antibiotics until the fever, localized closed infection, profuse discharge from the ulcer, or lymphadenitis clear; bed rest reduces the duration of the infection and aborts spread, which can result from the continuous trauma to the infected insensitive part. Thermography is a useful tool which permits early detection in the insensitive part of 'hot' areas resulting from infection or bruising, and possibly abortion of potential ulceration by the use of bed rest and, if necessary, appropriate antibiotics. It is also helpful in appreciating early joint involvement and can be used to regulate the therapeutic sequence, not only when to put the patient to bed but also when to allow him to be ambulant in a plaster cast.

Trophic ulceration should be treated initially with bed rest, soaks and antibiotics until the secondary infection is removed; bone sequestra, which significantly impair healing, should be excised. The patient may then become ambulant in a walking plaster cast. The plaster cast distributes the weight evenly, rests tissue, avoids the trauma of frequent dressing changes, and limits oedema by encapsulation. Covering the clean ulcer with fresh or lyophilized pigskin followed by a split skin autograft is used to reduce morbidity.

The tendency to blister formation may be reduced by wearing new shoes for a maximum period of 2 hours, relatively frequent changing of shoes, minimizing the wearing of shoes, avoiding long walks, resting feet frequently, and wearing two pairs of socks (a light and a heavy pair) so that the rubbing occurs between the outer and inner sock.

The main emphasis of treatment is that of accommodation to the existing problems rather than correction although attempts should be made to get the foot back to a neutral position to provide better accommodation. In patients with deformities resulting from accompanying motor paralyses, surgical correction may be necessary to afford the foot a greater ability to accommodate.

Once the physical insult from the forces of friction, shear and pressure to the foot has been removed, proper footwear is necessary to prevent its recurrence. In the making of shoes, material that is soft and capable of being moulded for total contact with the feet, and a rocker bar that forms a bulge behind the forefoot and protects the foot on its push-off and roll-over in walking, are desirable and necessary. Plastazote, a polyethylene micropore material, has been useful in manufacturing shoes and sandals for the insensitive foot. It can be heated and moulded easily, bottoms out (wearing out at pressure sites) late, and has memory, that is, it can be reheated and remoulded. A nylon covering on top of the plastazote would help reduce the stress of shear or friction. The use of microcellular rubber material is being investigated.

The basic steps in the making of shoes or their modification for insensitive feet are the administration of the footprint test which utilizes the Harris footprint mat and exposes the areas of the feet receiving abnormal pressures on standing and stepping (Fig. 5), the moulding of the soles or shoes to distribute weight bearing diffusely, and the administration of the 'inside the shoe footprint test', the technique of which is the same as the regular footprint test except that the Harris mat and paper are cut to conform to the shoe size (Fig. 6). The Harris footprint mat is a rubber mat which is made up of thousands of ridges of three different heights. The mat is inked, and a piece of paper is placed over it. When a person stands or steps on it, the amount of pressure each small area of foot carries can be

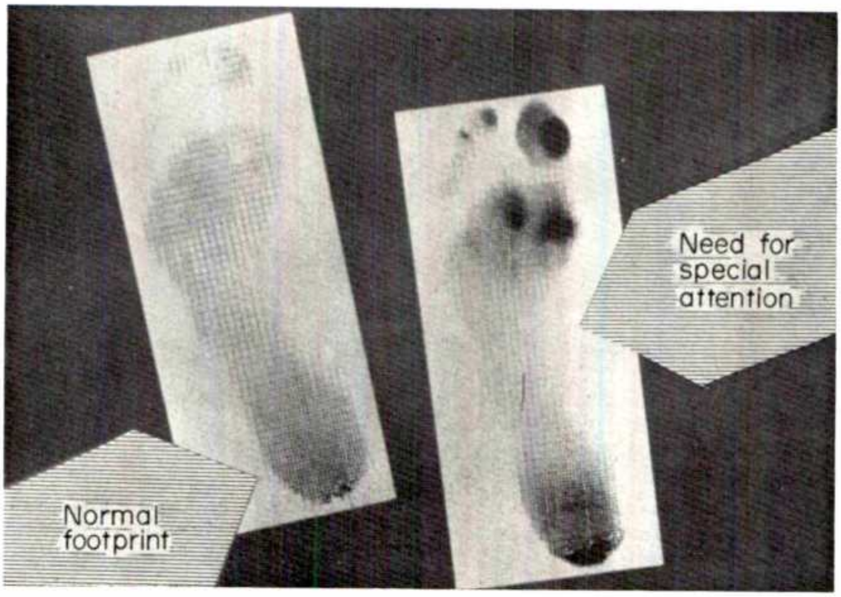

FIGURE 5. Using Harris footprint mat technique to assess the distribution of weight to the foot demonstrated 'hot' points, the comparatively darker areas.

determined (Drury *et al.*). Finally, the patient has to be educated about the problems of new shoes and to be given a description of the procedure for the 'breaking in' of new shoes.

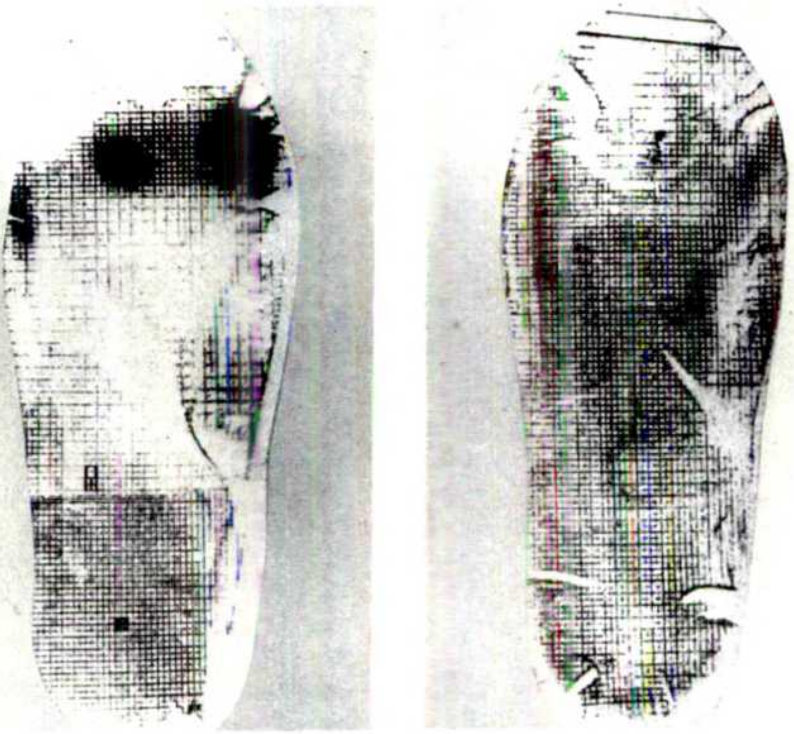

FIGURE 6. The same Harris footprint mat technique is used to assess distribution of weight to the foot in the shoe before and after correction

ACKNOWLEDGMENTS

I would like to acknowledge the assistance of, and the use of Figs. 4 and 6 from, Paul W.Brand, C.B.E., F.R.C.S., F.A.C.S. and Carl Enna, M.D.; and the use of Fig. 5 from William Crutcher, M.D. from the U.S. Public Health Service Hospital, Carville, Louisiana.

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Temperature dependence of water content of stratum corneum

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Accepted for publication 21 November 1974

SUMMARY

The water content of human stratum corneum has been measured gravimetrically *in vitro* in relation to relative humidity (RH) and temperature. Water content increased with increasing RH. Stratum corneum water content increased 50% when the temperature was raised from 20 to 35°C at RH below 60%. Temperature dependence decreased with increasing RH until there was essentially no temperature dependence at 90% RH. The conclusion is that temperature changes could significantly affect water content *in vivo* and pliability of skin at RH below 60%.

Previous work has indicated the importance of water content of the skin in protecting the body from environmental assaults including water, ultraviolet exposure, detergents and chemical irritants (Blank, 1952; Singer & Vinson, 1966). Water content of stratum corneum has been defined as a function of relative humidity (Singer & Vinson, 1966). We have studied the relationship between water content of stratum corneum and changes in temperature *in vitro* and report a previously undescribed temperature dependence of water content.

Blank (1952) showed that water content of stratum corneum varied with relative humidity (RH) and that the pliability of skin was reduced at RH below 60%. He estimated that the critical point for pliability was about 10 mg of water per 100 mg of dry stratum corneum. In general, he observed that water loss from stratum corneum increased at high temperature, at low humidity, and in flowing air. Clinical studies by Gaul & Underwood (1951) supported these observations and showed that chapping could be related to dew point which reflected temperature and humidity. Later work by Singer & Vinson (1966) indicated that water content of stratum corneum varied with RH in a logarithmic relationship and did not depend on absolute humidity.

Middleton & Allen (1973) proposed that the ability of stratum corneum to stretch prior to breaking is significantly less at lower temperatures. Wildnauer, Bothwell & Douglass (1971) demonstrated that decreased water content of stratum corneum has the same effect of reducing extensibility. Although

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Middleton discounted water content as a major factor in his tests, the present report shows that water content of stratum corneum is reduced at lower temperatures and might contribute to the loss of extensibility observed by him at lower temperatures.

MATERIALS AND METHODS

Stratum corneum from the abdominal region of cadavers was separated by trypsinization (Kligman & Christophers, 1963). Remaining epidermal tissue was gently removed from the membrane with a wool-tipped stick and the stratum corneum was stored over Drierite in a desiccator. Six mm diameter disks were cut from samples with a biopsy punch and were mounted on nichrome wire hooks. Hydration measurements were made in a 15 × 15 × 20 cm plastic chamber with a controlled environment. Within the chamber, up to six stratum corneum samples suspended from hooks were weighed on a balance wire extending down from a Cahn RG Electrobalance on top of the chamber. Transfer of samples to and from the balance wire was made by a slidewire inside the chamber so that all measurements were made without disturbing the environment of the chamber.

Humidity within the chamber was maintained by salt solutions which generate a constant RH from 10 to 35°C (Young, 1967). The following saturated solutions were used: magnesium chloride, (RH = 30%), potassium carbonate (40%), sodium nitrite (60%), sodium chloride (75%), potassium chloride (83%), potassium nitrite (93%), and potassium sulphate (96%). The hydration chamber was mounted within a modified Hydro-Jac Incubator (Forma Scientific), which was coupled with a water bath to regulate the temperature of the incubator (±0·2°C). Temperature was monitored with a 46 TU Telethermometer (Yellow Springs Instrument Co.), and humidity with Hydrodynamics narrow-range sensors located within the chamber. Changes in humidity and weight gain of samples were recorded simultaneously throughout the experiment.

Four samples from the same source were monitored on each run. Initially, samples were hung in the chamber and equilibrated over Drierite for at least 72 h. The dry weight of each sample was taken within the chamber at 20°C. The chamber was then opened and Drierite was replaced with salt solution. The closed system was allowed to equilibrate for 72 h at which time the weight had been stable for at least 24 h. Samples were then weighed within the chamber and the temperature was raised. Again, samples were equilibrated for 72 h and weighed. This procedure was repeated for the humidities specified at 10, 20, 30 and 35°C.

Maximum hydration was determined by hydrating samples for 24 h over distilled water at 30°C until equilibrium was reached. Then warm water was placed in the sample chamber, raising the RH to 100%, when the sample gained weight due to condensation. After equilibrium at 30°C for a second 24 h, a steady state was reached at a measured 97% RH and 92% weight gain. In previous studies with hydration at 97·5% RH, hydration had ranged between 90 and 106% weight gain for normal samples. Hence 92% hydration was taken as the maximum equilibrium weight gain in this study.

Hydration was monitored for ascending and descending temperatures for some samples, and no differences in the temperature dependence were observed. The observed hysteresis effects were small and were included in the data. Weight gain of samples was recorded as percent weight gain or mg of water per 100 mg of dry stratum corneum.

RESULTS

Fig. 1 shows % weight gain or water content of stratum corneum in relation to temperature and RH. Above 20% RH, mean water content of stratum corneum increases linearly with RH up to 60% RH, then rises exponentially up to 95%.

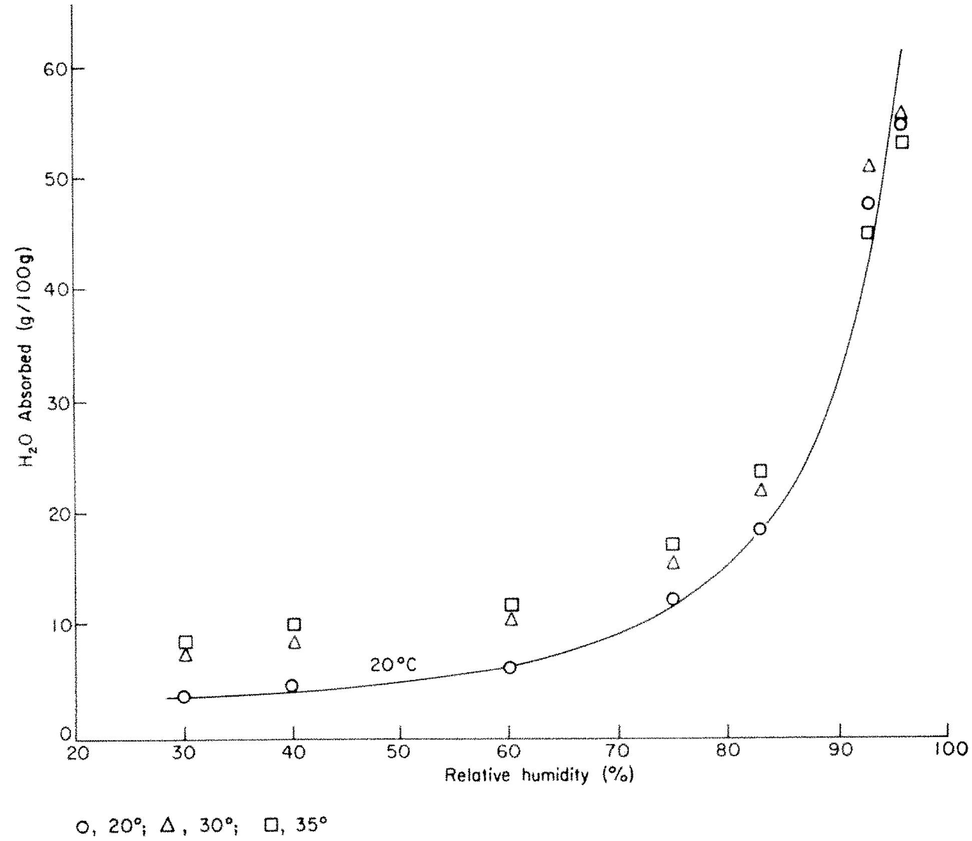

FIGURE 1. Hydration of stratum corneum as a function of relative humidity. Investigated for 20, 30 and 35°C.

Water content of stratum corneum samples can be discussed in terms of the equilibrium constant K associated with the hydration reaction:

$$\text{Dry stratum corneum} + H_2O \rightleftarrows \text{Hydrated stratum corneum.} \tag{1}$$

The equilibrium constant then becomes:

$$K' = \frac{[\text{Hydrated stratum corneum}]}{[\text{Dry stratum corneum}]\,[H_2O]}. \tag{2}$$

In the present work RH and, therefore, water concentration in air can be considered constant; hence, $[H_2O]$ can be included in K′ as $K = K'\,[H_2O]$. Dry stratum corneum can be described as the number of sites available for hydration (total sites) minus the number of sites occupied by H_2O (hydrated sites). Thus,

$$K = \frac{[\text{Hydrated sites}]}{[\text{Total sites} - \text{Hydrated sites}]}. \tag{3}$$

Sites occupied by H_2O per mg sample can be expressed as % hydration or mg (H_2O)/100 mg dry stratum corneum. Taking the extent of hydration at 97% RH as the maximum equilibrium hydration* of undamaged stratum corneum in the presence of water vapour as discussed in the methods section, total sites can be expressed as 92% hydration and

$$K = \frac{\%\ \text{hydration}}{92 - \%\ \text{hydration}}. \tag{4}$$

Temperature dependence is then reflected in the thermodynamic quantity enthalpy (ΔH°) which is related to K by the equilibrium expression for free energy (ΔG°) at constant pressure (Moore, 1962),

$$\frac{d\Delta G^\circ}{d(1/T)} = \frac{d\ \ln K}{d(1/T)} = \frac{-\Delta H^\circ}{R} \tag{5}$$

where R is the gas constant under conditions of constant pressure. Integration yields

$$\ln K = \frac{-\Delta H^\circ}{R}(1/T) + C. \tag{6}$$

C is a constant of integration which includes the constant H_2O concentration in air as well as entropic factors. Over the small temperature range in this study, ΔH° can be assumed constant, and lnK is plotted versus 1/T with slope $-\Delta H^\circ/R$ (Fig. 2). The r-correlations for all plots of lnK are significant ($\alpha < 0{\cdot}05$) with respect to 1/T, except for 1 run at 60% RH ($\alpha < 0{\cdot}10$) and runs at 93 and 95% RH in which the slope approaches zero. The range of K values for each RH is also indicated (Fig. 2). The enthalpies determined from plots like those in Fig. 2 are plotted in Fig. 3 as a function of RH. Ranges shown are 95% confidence limits.

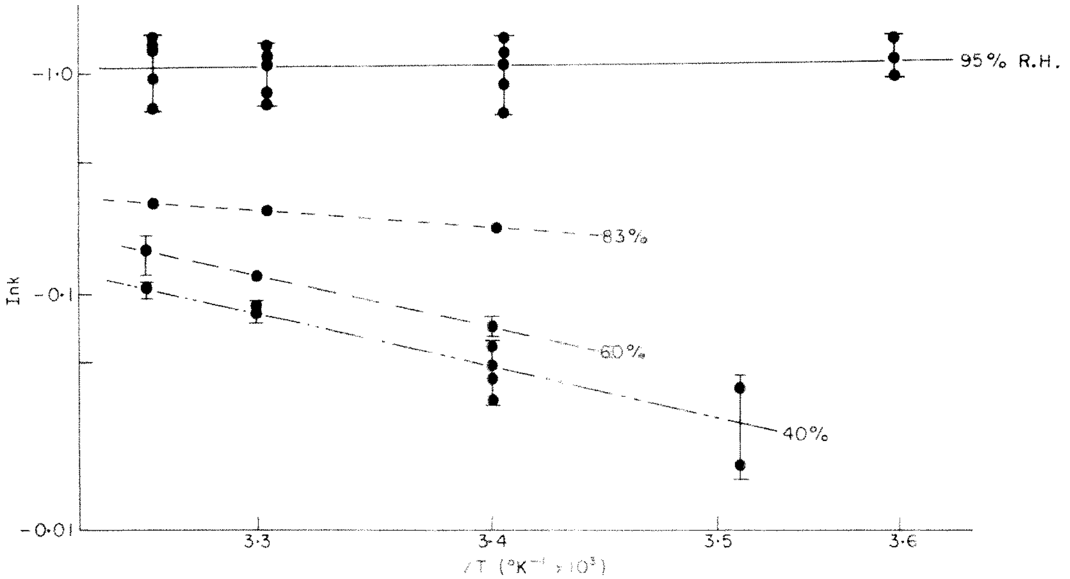

FIGURE 2. Stratum corneum hydration equilibrium constant K as a function of 1/T at four relative humidities. Range of values showing inter-sample variation indicated by vertical bars. Slope = $-\Delta H^\circ/R$.

[*] Varying the estimate of maximum hydration does not have a significant effect on the relation between k and temperature. Changing 92 to 60% changes ΔH less than the confidence limits shown in Fig. 3.

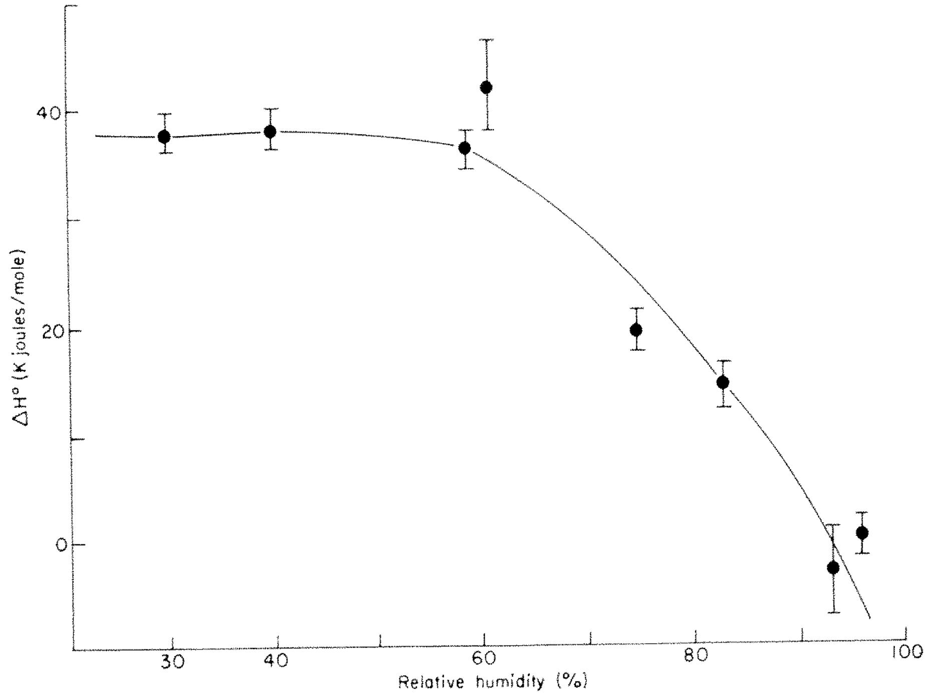

FIGURE 3. Enthalpy (ΔH°) of stratum corneum hydration equilibrium with 95 % confidence limits indicated by vertical bars.

DISCUSSION

The enthalpy (ΔH°) associated with the stratum corneum–water equilibrium describes a temperature dependence of water content at lower RH. At RH below 60%, stratum corneum rapidly loses its ability to retain water with decreasing temperature (Fig. 1 and Fig. 3). Although information on entropy is necessary before statements can be made concerning the overall heat of reaction, temperature dependence of hydration of stratum corneum is greater below 60% RH than at higher RH (Fig. 3). This indicates that the nature of the bonds broken by water in the hydration process changes from low to high relative humidities. The first type of bond might correspond to water molecules hydrating strong higher-ordered bonds between stratum corneum protein molecules to give skin its pliability. As Blank (1952) indicated, this necessary water is approximately 10% at normal skin temperature (Fig. 1). As RH increases, hydration above 10% involves water molecules which are bound less tightly (Scheuplein & Morgan, 1967). The change in ΔH° for hydration (Fig. 3) reflects a progressive change in the nature of the binding of water molecules as the stratum corneum absorbs more water. This additional hydration at RH above 60%, however, is not necessary for normal pliability of skin.

In chapping and dry skin conditions, consideration must be given to the ability of skin to retain the 10% water necessary for pliability and extensibility. At 60% RH and 30°C, water content of stratum corneum is only slightly above 10% (Fig. 1), decreasing to approximately one-half this value at 20°C. During colder winter months when the incidence of chapping increases (Gaul & Underwood, 1951), stratum corneum could reasonably reach a surface temperature of 20°C or below when exposed to cold air. Lower temperature and lower relative humidity in winter could decrease the ability of stratum corneum to retain water, thereby reducing its pliability. Thus, the observed relationship between temperature and water content of stratum corneum should be considered along with changes in mechanical properties and environmental conditions as causes of dry skin.

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British Journal of Dermatology (1975) **93**, 165.

Intraendothelial tubular aggregates in experimental wounds

ULTRASTRUCTURAL STUDY

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Accepted for publication 18 November 1974

SUMMARY

Intraendothelial tubular aggregates were found in small dermal blood vessels in three healing superficial wounds in patients receiving systemic corticosteroids. Similar inclusions were also demonstrated in two wounds in healthy subjects, but were not found in normal (unwounded) skin of either healthy subjects or steroid-treated patients.

Tubular aggregates of similar structure have previously been described as occurring in 'connective tissue' or 'auto-immune' diseases, including lupus erythematosus and dermatomyositis, both in the skin and in internal organs.

Although it has been suggested that such aggregates are of viral origin, their morphogenesis and significance remain undetermined. Our findings do not support a viral cause, but suggest that these structures are probably a product of regenerating endothelium.

Several recent ultrastructural studies have revealed submicroscopic intracytoplasmic tubular structures in various tissues in human and animal disease. In comprehensive reviews emphasizing the widespread occurrence of these structures, they have been referred to as 'tubular arrays' (Uzman, Saito & Kasac, 1971) and 'lupus-type inclusions' (Prunieras, Grupper & Durepaire, 1974). They have been demonstrated most commonly in the 'connective tissue' or 'auto-immune' diseases, and particularly in the skin and kidney in systemic lupus erythematosus (SLE). Some authors have suggested that these structures may be of diagnostic value in SLE (Bloodworth & Shelp, 1970; Györkey *et al.*, 1972; Prunieras *et al.*, 1972; Haustein, 1973 a, b), and in dermatomyositis (Prunieras *et al.*, 1972; Landry & Winkelmann, 1972).

The origin of the cytoplasmic inclusions remains undetermined. It has been suggested, on morphological grounds, that they are viral or virus-like (Chou, 1967; Györkey *et al.*, 1969; Kawano, Miller & Kimmelstiel, 1969; Hashimoto & Chandler, 1972) but the evidence is inconclusive. Immunological studies in SLE did not show specific myxovirus antigens in involved tissues (Norton, 1969; Pincus

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et al., 1970) or elevated serum levels of antimyxovirus antibodies (Norton, 1969). Similarly, virological studies by Feorino, Hierholzer & Norton (1970) failed to provide evidence of an infectious agent. In a study of dermatomyositis in which tubular aggregates were found, tissue culture studies and serological tests for several paramyxoviruses were negative (Hashimoto *et al.*, 1971). A further view is that the inclusions are a cellular reaction product, following injury (Norton, 1969; Haas & Yunis, 1970; Fraire *et al.*, 1971; Uzman *et al.*, 1971). Hurd, Eigenbrodt & Ziff (1969) suggested that they represented material, released into the circulation following cellular damage, which is then phagocytosed by endothelial cells.

In this communication we report new observations of intraendothelial aggregates in microvessels of healing experimental wounds, found in the course of a systematic electron microscopic analysis of the effects of systemic corticosteroid therapy on wound repair in human skin (Eady & Odland, 1974).

MATERIALS AND METHODS

Four adult patients (2 male and 2 female), receiving long-term systemic glucocorticoid treatment for endogenous asthma, agreed to act as experimental subjects. The minimum treatment period was 10 years, and the dose range was equivalent to 10–60 mg prednisolone per day at the time of the experiment. Three patients had secondary changes in the skin attributable to steroid therapy, including atrophy and purpura. Otherwise, none had skin disease.

Series of linear superficial skin incisions were made in the flexor surface of one forearm of each patient, in the manner previously described (Odland & Ross, 1968). After intervals of between 1 and 21 days, the healing wounds were biopsied without anaesthesia, using a high speed power-driven punch of 2·5 mm diameter. Normal (unwounded) skin of the contralateral forearm was biopsied for control purposes. Similar wounds in four healthy young adult volunteers were also examined.

The biopsy specimens were fixed in chilled 1·33% osmium tetroxide in S-collidine buffer (pH 7·35) for 2 h. Primary fixation of a few specimens was carried out in a glutaraldehyde–formaldehyde solution buffered with sodium cacodylate at room temperature for 4 h.

After dehydration in ethanol, the tissues were embedded in Epon 812. Ultrathin sections were cut with diamond knives on a Reichert OmU2 ultramicrotome, collected on coated grids, stained with uranyl acetate followed by lead citrate (Reynolds, 1963) and examined in an AEI EM6 B electron microscope.

Two blocks were obtained from each biopsy specimen, and several sections from each block were examined. Twenty to thirty five endothelial cells per section were examined.

RESULTS

Between 5 and 14 days after wounding, tubular aggregates were found in endothelial cells of small dermal blood vessels related to the wound bed. They occurred principally in capillaries but occasionally in venules and arterioles. Tubular aggregates were revealed in three of nine wound biopsies from the steroid-treated patients, and in two of five wound biopsies from normal subjects.

No attempt was made to quantify the findings, as the density of blood vessels varied considerably in the specimens. In general, however, tubular aggregates were not abundant. Usually one, and not more than two aggregates were present in individual endothelial cells (Fig. 1). They were located in the cytoplasm and were partially or wholly enclosed by a single membrane which was usually smooth, but occasionally studded with ribosomes, and which appeared to be part of or directly related to endoplasmic reticulum (Figs 2 and 3). The aggregates were composed of several convoluted and branched components, usually appearing tubular in cross section. A dense layer, approximately

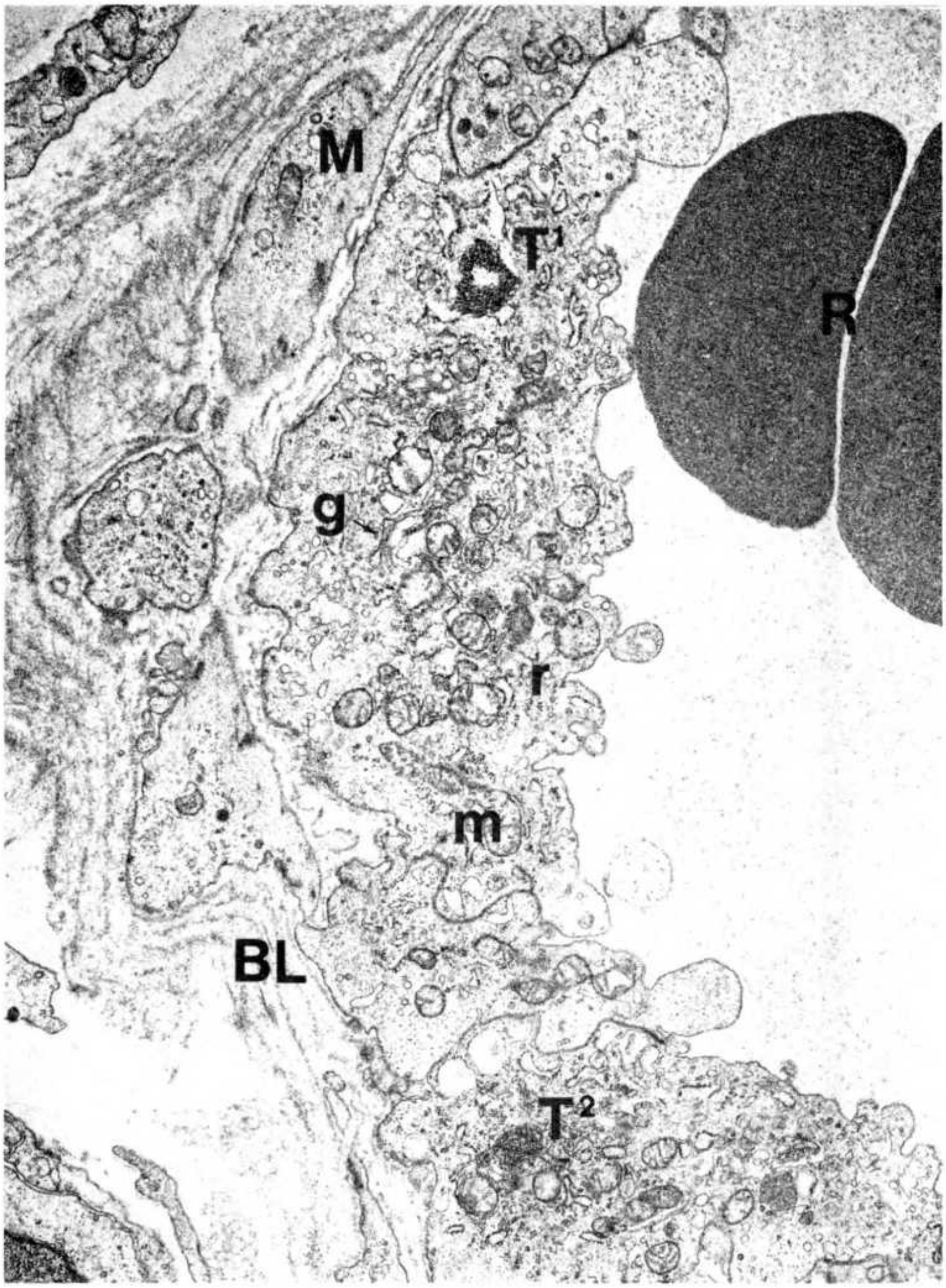

FIGURE 1. Tubular aggregates (T1 and T2) are demonstrated in different endothelial cells of a venule. Note abundance of cytoplasmic organelles including Golgi complex (g) mitochondria (m) and granular endoplasmic reticulum (r). Also shown are red blood cells (R) in vessel lumen, multi-layered basal lamina (BL) and smooth muscle (M). (× 13,500.)

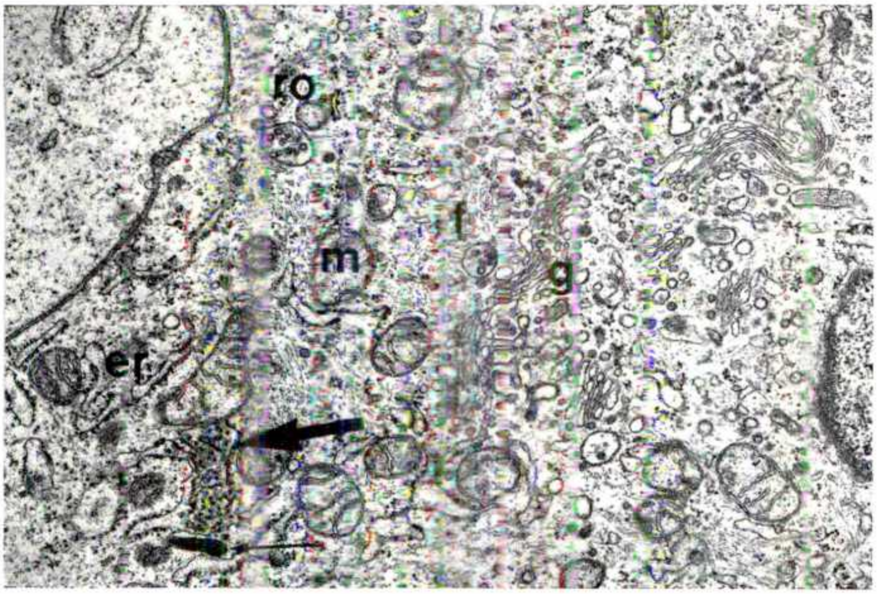

FIGURE 2. Portion of endothelial cell containing tubular aggregate (large arrow) which appears to be enclosed in dilated granular endoplasmic reticulum. Note that cytoplasm is densely packed with mitochondria (m), endoplasmic reticulum (er), free ribosomes (ro), extensive Golgi complex (g): probably features of cell regeneration. f, filaments; small arrow indicates endothelial granule. (× 16,000.)

60Å thick, surrounded a less dense core or lumen (Fig. 3a). In some instances, the electron lucent core could not be seen. The outside diameter of the tubules ranged from 190Å to 240Å. In several tubules there was a fine cross striation of electron dense and less dense bands (Figs 3a and 3b) of 65Å periodicity. In one aggregate (Fig. 3b) a fine dense lamella or septum, about 40Å thick, was seen midway between the outer dense layers of numerous tubules. At several points the outer dense layer appeared to be continuous with the membrane surrounding the aggregate.

Essentially, there was no difference between the aggregates in wound tissue from the steroid-treated and from normal subjects; however, the fine internal structure of the tubules in Fig. 3(b) was resolved only in one aggregate which was in tissue from a steroid treated subject. No tubular aggregates were seen in blood vessels of unwounded (control) skin.

Several of the endothelial cells containing aggregates had abundant cytoplasmic organelles including mitochondria, ribosomes (occurring individually or aggregated as polysomes), and rough endoplasmic reticulum. There were relatively few pinocytotic vesicles. Frequently there was multilayering of the basal lamina surrounding endothelial cells and pericytes (Fig. 1).

DISCUSSION

As we have found the structures described in this study only in endothelial cells, we prefer to designate them as intraendothelial tubular aggregates (ETA) instead of the more general term 'cytoplasmic inclusions'. Morphologically, they correspond closely to ETA of other reports (see Table 1) but it should be mentioned that similar aggregates have been found in different cell types, including lymphocytes (Prunieras *et al.*, 1971; Jensen *et al.*, 1971; Haustein, 1973a; Goodman *et al.*, 1973;

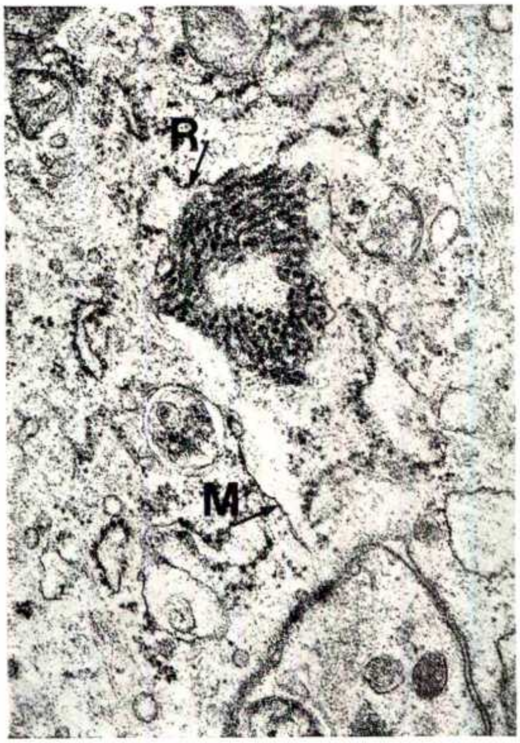

FIGURE 3(a). Higher magnification of tubular aggregate (T1) shown in Fig. 1. Tubular profiles are demonstrated in cross-section; in longitudinal section, individual tubules exhibit cross striations. Aggregate is partly surrounded by single membrane which is smooth (M) and studded with ribosomes (R). (× 36,000.)

Klippel, Grimley & Decker, 1974) fibroblasts and macrophages (Norton, Velayos & Robison, 1970; Nieland, Hashimoto and Masi, 1972; Haustein, 1973a) and keratinocytes (Nieland *et al.*, 1972; Haustein, 1973a). We are unaware of a previous account of tubular aggregates in endothelium or other tissues in healing wounds.

Details of the fine morphology of these tubular structures vary in different reports and for this reason alone we must be cautious in assuming that ETA in our study are identical with those in other systems. However we realize that in the interpretation of morphological parameters (e.g. diameter of the tubules) allowance has to be made for differences in preparative techniques for electron microscopy. A new ultrastructural feature which we may have discovered is the presence of a septum or lamella in the lumen of some tubules. As this feature was seen in only one aggregate, the significance of this finding has still to be assessed, as well as its incidence.

ETA are found most commonly in 'connective tissue' or 'auto-immune' disease, SLE being the most prevalent. It is notable that in all these conditions angiopathy of small vessels is an important feature. The complex nature of the angiopathic processes is as yet poorly understood, but some evidence points to an immunological basis. However, other causes of vessel damage could play a role. Although there is a remote possibility that a latent virus infection could affect the steroid-treated subjects in this study, none of the group had overt viral infection. There is no evidence to implicate a viral or

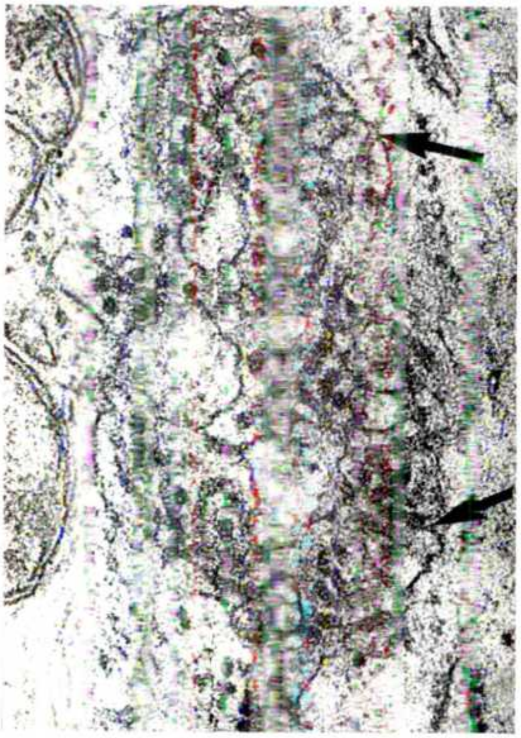

FIGURE 3(b). Membrane forming walls of tubules are seen at points (arrows) to be in direct continuity with membrane of endoplasmic reticulum. Central dense lamellae are visible in some tubules. (× 110,000.)

other infectious agent as the cause of ETA which were found only in wounds and not in control (unwounded) skin. The work of Baringer & Swoveland (1972) supports the view that the tubules originate as invaginations of endoplasmic reticulum membrane and are thus most probably a host cell product rather than a viral inclusion.

Our interpretation is that ETA are a consequence of endothelial damage or regeneration following injury as performed in this study. However, this hypothesis cannot account for the pathogenesis of tubular aggregates in all systems; for example, in umbilical cord blood lymphocytes from infants of mothers with SLE, the possibility of a humoral mode of induction of the aggregates has been suggested (Klippel *et al.*, 1974).

In their description of regenerating blood vessels in healing wounds, Schoefl & Majno (1964) mentioned that endothelial cells possess greater numbers of the usual organelles including ribosomes, mitochondria and endoplasmic reticulum, but fewer vesicles. We found that most endothelial cells containing ETA also had abundant organelles possibly reflecting the regenerative process. It is notable that in many reports on ETA, little or no mention is given to general cytological features of endothelial cells. There are certain exceptions, however. In a study of dermatomyositis, Landry & Winkelmann (1972) described cytoplasmic changes in 'inclusion-positive' cells, including increased numbers of various organelles. These cells 'resembled young proliferative, transforming, and undifferentiated cells' whereas others 'resembled degenerating or de-differentiating cells. . .' In lupus erythematosus, Haustein (1973a) described cytological features of ETA-containing endothelial cells which were quite

TABLE 1. Published reports on intraendothelial tubular aggregates

Organ	Disease	Authors
Skin	Lupus erythematosus	Norton (1969); Haas & Yunis (1970); Prunieras *et al.*, (1970); Garancis *et al.* (1971); Nieland *et al.* (1972); Haustein (1973a); Berk & Blank (1974)
	Scleroderma	Prunieras *et al.* (1972); Györkey *et al.* (1972)
	Dermatomyositis	Norton *et al.* (1970); Prunieras *et al.* (1971); Hashimoto *et al.* (1971);
		Hashimoto *et al.* (1971); Landry & Winkelmann (1972); Prunieras *et al.* (1972)
	Warts, Bowen's disease, Basal cell epithelioma	Maciejewski *et al.* (1973)
	Degos' disease	Nishida & Howard (1968)
	Pemphigoid	Macadam, Vetters & Saika (1975)
Kidney	Lupus erythematosus	Györkey *et al.* (1969); Hurd *et al.* (1969); Kawano *et al.* (1969); Norton (1969); Haas & Yunis (1970); Pincus *et al.* (1970); Bloodworth & Shelp (1970); Tisher *et al.* (1971); Garancis *et al.* (1971); Haustein (1973b)
	Scleroderma	Norton (1969); Györkey *et al.* (1972)
	Goodpasture's syndrome	Norton (1969)
	Idiopathic thrombocytopenic purpura	Györkey *et al.* (1969)
	Sjögren's syndrome	Shearn *et al.* (1970); Györkey *et al.* (1972)
Lung	Lupus erythematosus	Fraire *et al.* (1971)
Liver	Lupus erythematosus	Kovacs, Horvath & Warren (1972)
Muscle	Dermatomyositis	Prunieras *et al.* (1971); Hashimoto *et al.* (1971); Norton *et al.* (1970)
	Lupus erythematosus	Norton (1969)
	Polymyositis	Györkey *et al.* (1972)
Brain	Herpes simplex encephalitis	Baringer & Swoveland (1972)
Synovium	Rheumatoid arthritis	Györkey *et al.* (1972)

different from our observations in wounds. In his study, endothelial cells contained an increased number of multivesiculated and diffusely dense bodies (lysosomes), multiple pinocytotic vesicles, myelin figures and densely packed filaments—all features that may be interpreted as reflecting the degenerative rather than regenerative process.

ETA have been found in clinically normal skin of patients with SLE (Garancis *et al.*, 1971; Nieland *et al.*, 1972; Haustein, 1973a) and with dermatomyositis (Prunieras *et al.*, 1971) but we are not aware of reports of ETA in healthy subjects. Finding ETA in experimental wounds in healthy subjects in this study, therefore, may be of particular significance. The relationship between corticosteroid therapy and ETA has yet to be determined. Certainly most of the conditions associated with ETA are treated with corticosteroids but various authors mention that ETA occur irrespective of whether corticosteroid treatment has been given or not (Garancis *et al.*, 1971; Tisher *et al.*, 1971; Nieland *et al.*, 1971; Haustein 1973 a, b). A comparison between the relative frequency of ETA in wounds of normal subjects and those in steroid-treated patients has not been attempted because of the limited number of subjects investigated. Obviously it would be of considerable interest to know the incidence of ETA in regenerating blood vessels in wound healing in normal subjects. A further study of this problem is underway.

ACKNOWLEDGMENTS

We are grateful to Mary Hoff, Pamela Joseph and Jerry Krivacek for technical assistance. This work was supported in part by U.S.P.H.S. research grants AM 08368 and GM 16598. Dr Robin Eady is a recipient of a Wellcome Trust Dermatological Research Fellowship.

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British Journal of Dermatology (1975) **93**, 175.

Long-spacing collagen in skin biopsies from patients with lepromatous leprosy

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Accepted for publication 25 November 1974

SUMMARY

In the course of evaluating the effect of different drugs on the morphology of *Mycobacterium leprae* (Edwards, Draper & Draper, 1972) and on the phagocytic cells containing these organisms (Edwards, 1973), an unusual feature was observed in the dermal connective tissue of skin biopsies obtained from some of the patients. Variable amounts of an extracellular, cross-striated osmiophilic material were present in the dermis.

The purpose of this communication is to describe the banded substance observed in the extracellular space of the lepromatous skin and to compare it briefly with similar substances and structures reported to occur elsewhere.

MATERIALS AND METHODS

The material for the present study consisted of thirteen skin biopsies obtained in two series (A & B) from six patients with leprosy.

Series A

Two biopsies were obtained from two patients prior to treatment for 1 year with 4, 4′-diaminodiphenyl sulphone (DDS) (600 mg per week); at the end of the year each patient was biopsied again. The tissues were fixed in buffered formaldehyde for light microscopy and post-fixed in osmic acid for electron microscopy.

Series B

A total of nine biopsies were obtained from four patients as follows: three biopsies from one patient (prior to and following 3 and 6 weeks treatment with rifampicin, 600 mg daily); three biopsies from a second patient (prior to and following 3 and 6 weeks treatment with DDS, 100 mg daily on 6 days per week); two biopsies from a third patient (prior to and following 6 weeks of treatment with streptomycin, 1 g daily), and one biopsy from a fourth patient following 6 weeks of treatment with thiambuto-

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sine, 1 g weekly by injection. From this series the biopsies were bisected; one half was fixed for light microscopy in 4% neutral formalin and the other was prefixed in 2·5% glutaraldehyde in 0·085 M-cacodylate buffer, at pH 7·4 (Glauert & Thornley, 1966), for electron microscopy. Tissues prefixed in glutaraldehyde were washed in 7·5% (w/v) sucrose in cacodylate buffer at pH 7·4 and containing 0·1 M calcium chloride. All tissues for electron microscopy (from series A and B) were fixed in 1% OsO_4 in veronal buffer (Kellenberger, Ryter & Séchaud, 1958). Following fixation the tissues were embedded in Araldite and sectioned on an LKB Ultratome III. Sections 1 μm thick were stained with toluidine blue. Ultrathin sections were stained with saturated aqueous uranyl acetate and lead citrate and examined in a Siemens Elmiskop 101.

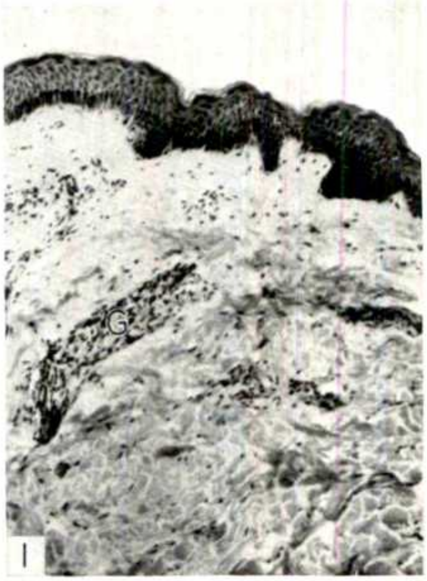
All tissues fixed for light microscopy were processed by conventional techniques, embedded in paraffin, and 5–7 μm thick sections were stained by the Triff method (Wheeler, Hamilton & Harman, 1965) for examination.

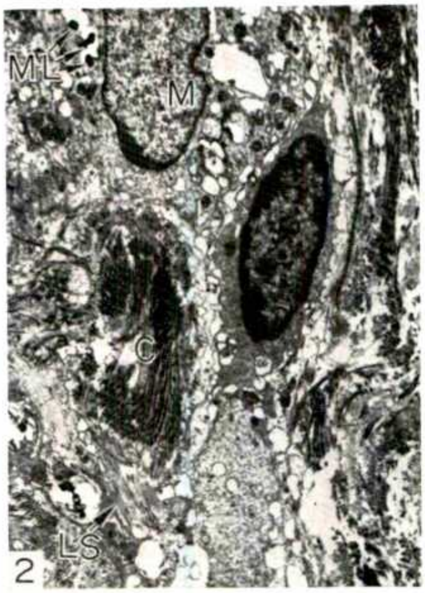
RESULTS

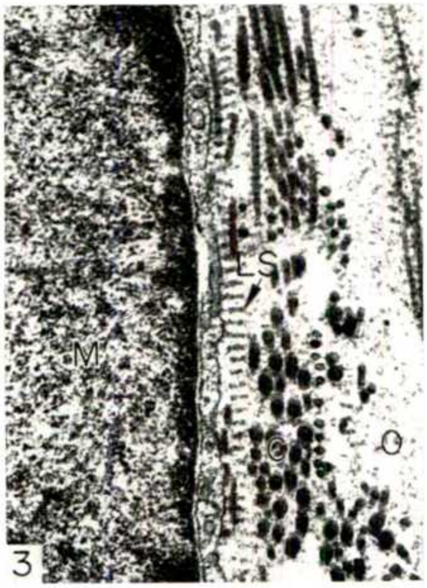
Skin biopsies taken from lepromatous patients had a thin epidermis from which the rete ridges had largely disappeared. The dermis was occupied by scattered areas of chronic inflammatory infiltrate amongst dense collagen fibres (Fig. [illegible]). The granulomata were made up largely of histiocytes and macrophages, as has been found also by Papadimitriou & Spector (1972), containing viable and non-viable *Mycobacterium leprae*, small numbers of fibrocytes, mast cells, plasma cells, lymphocytes, and

TABLE 1. Drug treatment of leprosy patients whose skin biopsies were examined for the presence of long-spacing collagen

Patient no.	Drug	Years after treatment	Amount of long-spacing collagen seen in electron micrographs
Series A			
16101	DDS	0	None
		1	Small
16052	DDS	0	None
		1	Small
Series B			
16308	Rifampicin	0	Small
		3	Small
		6	Large
16305	DDS	0	None
		3	None
		6	Small
16316	Streptomycin	0	None
		6	Small
16411	Thiambutosine	6	Small







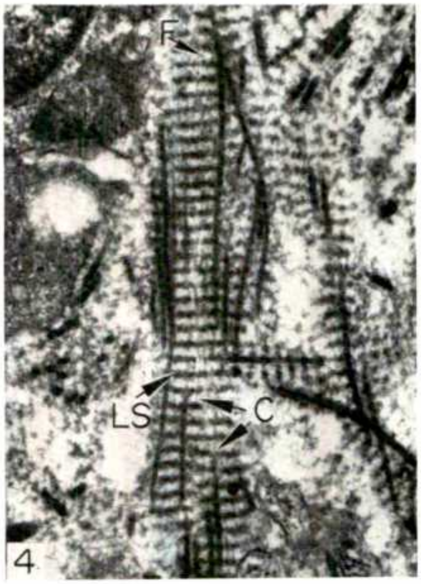


FIGURE 1. Skin biopsy from lepromatous leprosy patient showing granulomatous inflammatory infiltrate (G) in the dermis (× 45).

FIGURE 2. Electron micrograph illustrating cells of granulomatous inflammatory infiltrate amongst native collagen fibres (C) and long-spacing collagen (LS). *Mycobacterium leprae* (ML) in a macrophage (M). Skin biopsy 6 weeks after rifampicin treatment (× 5600).

FIGURE 3. Electron micrograph illustrating long-spacing collagen (LS) in close proximity to the cellular membrane of a macrophage (M); native collagen fibres (C) of varying diameter intermingle with the long-spacing collagen; flocculant material (O) is also present. Skin biopsy 6 weeks after rifampicin treatment (× 30,000).

FIGURE 4. Electron micrograph showing some details of long-spacing collagen (LS); fine fibrils connect the cross-striations (F); native collagen fibres (C) are in apparent continuity and intermingle with the long-spacing collagen; deposit of fine fibrillary and flocculant material is present in the background (× 30,000).

collagen fibres (Fig. 2). In addition, a broad-banded substance was observed in the extracellular space (Figs 3 and 4). This broad-banded material was not observed in electron micrographs in all the tissues; it was most abundant in the last of the three biopsies taken from the patient who was being treated with rifampicin. However, there was a certain amount of the broad-banded material to be seen in the biopsy from the same patient before treatment with rifampicin (Table 1). The broad-banded substance consisted of an osmiophilic, cross-striated material, which had a periodicity of approximately 1200Å, as compared with the periodicity of 640Å of the native collagen. No interperiod striations were observed in the banded substance, but very fine fibrils connecting the cross-striations were present (Fig. 4). A fine fibrillary and/or flocculant material, probably representing the ground substance, was also observed in the extracellular space. The deposition of the broad bands in relation to native collagen was variable; sometimes it was intermingled closely with native collagen fibres of various diameters, conveying a ladder-like appearance, and individual fibres of native collagen were in apparent continuity with the banded material (Fig. 4). In other locations the native collagen fibres were absent and the banded structures were short and randomly scattered. None of the banded substance seen had a fusiform shape. This material was observed often in the immediate vicinity of, and sometimes in close apposition to the cellular surface membrane of macrophages containing *Mycobacterium leprae* (Fig. 3).

DISCUSSION

The broad-banded substance observed in the granulomatous infiltrated areas in the skin biopsies from lepromatous patients was comparable with those reported by a number of workers in normal tissues, tumours, tissue cultures and other experimental material (Table 2). In normal tissues they were observed by Jakus (1956) in Descemet's membrane; by Cauna & Ross (1960) in Meissner's corpuscle; by Feeney (1962) in the trabecular region of the human eye; by Naumann & Wolfe (1963) in the subcommissural organ in rat brain; and by Silberberg, Silberberg & Feir (1963) in the articular cartilage of the mouse.

In tumours of the nervous system they were observed by Luse (1960), Ramsay (1965), Waggener (1966), Poirier, Escourolle & Castaigne (1968) and by Cravioto & Lockwood (1968).

In tissue culture of rabbit adenohypophysis, Chatterjee (1973) observed extracellular broad-banded structures, and Goldberg & Green (1964) observed them in fibroblast cultures.

In experimental material they were observed by Pillai (1964) in constricted nerves and by Edwards (personal observation, 1973) in dermal nerves following the removal of over-lying keratin.

In our material these banded structures had a periodicity of approximately 1200Å; they were observed either in continuity with the native collagen fibres or intermingled with a fine fibrillary material. With respect to these features they resemble similar structures reported in other studies. However, they differed in other aspects. Thus they were not seen as fusiform structures but as broad bands of varying length and width; there were no interperiod lines; and there was no spatial relation of this substance to cellular basement membrane, as was the case in the observations of the majority of workers quoted in Table 2. It has been generally assumed that these structures represent a variant form of long-spacing collagen, the approximate periodicity varying from 1000Å to 1400Å. At times the periodicity was reported to be from 770Å to 950Å; possibly, the latter structures may be another variant form.

It is known that tropocollagen molecules may be orientated differently from those of native collagen when factors in the micro-environment are changed. Long-spacing collagen has been obtained *in vitro* from native collagen extracted from tissues by experimental means (Highberger, Gross and Schmitt, 1951) and variant forms were produced by varying the experimental conditions (Randall, 1953). It

TABLE 2. Variant forms of long-spacing collagen observed in different tissues

Tissue	Periodicity approximate (Å)	Basement membrane in connection with	Form	Cells in immediate vicinity	Author	Date
Normal						
Descemet's membrane: calf and steer	1070	–	Long broad fibrils	Endothelial cell	Jakus	1956
Meissner's corpuscle: human	1200	+	Short and fusiform	Laminar cells	Cauna & Ross	1960
Trabeculum of eye: human	1000		Long and narrow also short	Endothelial cells	Feeney	1962
Sub-commissural organ of rat brain	1070	+	Short broad fibrils	Endothelial cells and sub-commissural cells	Naumann & Wolfe	1963
Articular cartilage: mouse	1200	–	(Tactoids) fusiform	Degenerating cartilage cells	Silberberg, Silberberg & Feir	1963
Muscle spindle: snake	double that of the collagen period	+	Long and narrow	Intrafusal fibres	Pallot & Ridge	1972
Muscle spindle: snake	1250	+	(Crescent)	Intrafusal fibres	Pallot & Ridge	1973
Experimental						
Experimentally constricted nerves: rat	1240	+	Fusiform and ribbon like	Schwann cells	Pillai	1964
Keratin removal from skin: dermal nerves: human	—	+	Fusiform	Schwann cells	Edwards	1973

TABLE 2 (*cont.*). Variant forms of long-spacing collagen observed in different tissues

Tissue	Periodicity approximate (Å)	Basement membrane in connection with	Form	Cells in immediate vicinity	Author	Date
Tissue culture						
Adenohypophysis: rabbit	950	—	Fusiform	Pituitary cells	Chatterjee	1973
Fibroblasts: mouse	850	—	Fusiform	Fibroblasts	Goldberg & Green	1964
Tumours						
Brain tumours: 8th acoustic: human	1200	+	Fusiform		Luse	1960
Brain tumours: human: acoustic neuroma	1040		Fusiform	Schwann cells	Raimondi, Mullen & Evans	1962
Von Recklinghausen tumour: human	1175	+	Fusiform	Schwann cells: fibroblasts	Ramsey	1965
Neuro-astrocytoma: human	1000	+		Endothelial and perivascular cells	Ramsey	1965
Astrocytoma grade 4: human	1100	—		Tumour cells	Ramsey	1965
Pinealoma: human	770			Fibroblasts	Ramsey	1965
Acoustic traumatic neuroma: human	1200	+	Fusiform	Schwann cells	Hilding & House	1965
Benign: sheath tumour: human	—	—	—	—	Waggener	1966
Von Recklinghausen neurofibroma: human	1400	+	—	Schwann cells	Poirier, Escourolle & Castaigne	1968
Acoustic nerve tumours (*in vivo* & *in vitro*): human	1200	+	Fusiform	Schwann cells	Cravioto & Lockwood	1968 1968
Nerve sheath neoplasm: human	1100	+	Fusiform	Schwann cells	Harkin & Reed	1969

was found by Robertson (1964) that many factors modified the morphology of native collagen *in vivo*; these included vitamin C deficiency, mucoproteins, polysaccharides and lipids, as well as endocrines (Priest, 1967).

In the granulomata present in lepromatous leprosy there are a number of factors in the micro-environment which also may cause tropocollagen molecules to orient themselves differently than do those in native collagen, particularly when the process is characterized by the presence of cells that lack basement membranes. The effect of drugs may also be an important factor in the formation of the altered collagen.

Thus, the formation of long-spacing collagen in living tissues is a phenomenon of considerable interest and worthy of further investigation.

SUMMARY

In the dermis of thirteen skin biopsies obtained from six leprosy patients, granulomatous inflammatory infiltrates were made up largely of: macrophages, with phagocytosed *Mycobacterium leprae* in various stages of degeneration; fibroblasts; plasma cells; mast cells and lymphocytes. Between these cells and intermingled with native collagen fibres (major periodicity: 640Å) broad-banded substance was observed with the electron microscope. It is considered to be a variant form of long-spacing collagen having a major periodicity of approximately 1200Å. It seems probable that the micro-environment in the granulomas is such that it promotes orientation of tropocollagen molecules different from that in native collagen. Several forms of long-spacing collagen, some with a similar periodicity, have been previously observed in normal tissues, cultures and tumours by other workers.

ACKNOWLEDGMENTS

The author wishes to thank Dr J.M.H. Pearson, British Medical Research Council Leprosy Research Unit, Sungei Buloh, Malaysia, for supplying skin biopsies and Professor Daria Haust, Department of Pathology, The University of Western Ontario, London, for invaluable help and advice.

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British Journal of Dermatology (1975) **93**, 183.

Hazards of steroid therapy in hepatic failure

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Accepted for publication 8 November 1974

SUMMARY

Three patients with extensive skin disease and liver failure are described. Two were treated with large amounts of topical corticosteroids and one with a small oral dose of betamethasone. The former two patients developed Addisonian crises when they failed to apply the topical steroids and all three patients developed aseptic necrosis of bone. This report emphasizes that the hazards of steroid therapy may be increased in patients with hepatic failure.

The complications of steroid therapy are well known, but it is less well recognized that the hazards may be greater in patients with hepatic failure. We here report three patients with skin disease and liver failure who developed serious systemic side effects as a result of either topical steroids or a small dose of systemic steroids.

CASE REPORTS

The three patients (F.C., R.M. and J.S.) were all between 45 and 55 years old. The first two had had extensive psoriasis with intermittent erythroderma for 30 and 8 years respectively. Neither patient could tolerate tar or dithranol applications and consequently had applied large quantities of undiluted fluorinated steroids (up to 100 g per day) for 3 to 4 years. The third patient (J.S.) had had constitutional eczema for 12 years which had not responded satisfactorily to topical steroids or tar. She had taken a small dose of betamethasone (0·5 mg twice daily) for 6 months before she developed aseptic necrosis of the femoral head.

All three patients were chronic alcoholics with hepatomegaly and two were persistently jaundiced. Patients F.C. and R.M. had some features of portal hypertension. Liver function tests are shown in Table 1. Patients F.C. and R.M. had the cutaneous complications of topical glucocorticoid therapy, purpura, striae and thin skin and they also had a buffalo hump and Cushingoid facies. On two occasions they each needed emergency admission because of malaise, drowsiness, hypotension, erythroderma and dehydration. Each of these episodes occurred several days after an alcoholic 'binge' when they had failed to apply topical corticosteroids for several days. The diagnosis of adrenal insufficiency was confirmed by the plasma electrolytes and cortisol (Table 1). It was necessary to

TABLE 1. Liver function tests

Patient	Bilirubin (mg%)	Proteins (albumin/globulin ratio)	SGOT (u/ml)	Alkaline phosphatase (K.A. units)	Sodium* (mmol/l)	Potassium* (mmol/l)	Cortisol* (μg/ml)
F.C.	0·5	↓ albumin	48	21	128·0	6·2	1·4 1·3 0·4
R.M.	1·8	↓ albumin	57	61	124·0	6·7	— — —
J.S.	1·7	Normal	40	10	—	—	— — —

* Samples taken during Addisonian crisis.

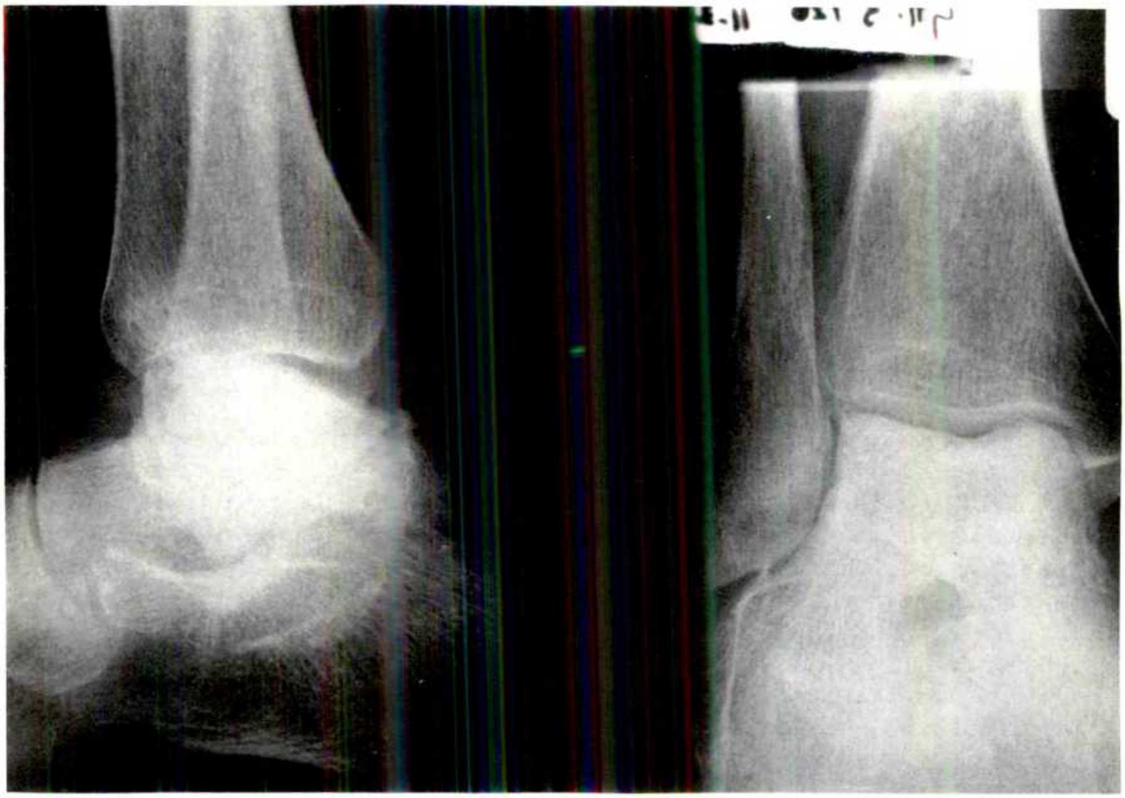

FIGURE 1. Aseptic necrosis of the talus in patient R.M.

treat the patients with cortisone acetate 25 mg twice daily for 2 weeks to control the Addisonian crisis. The skin was treated with topical fluorinated corticosteroids and after 2–3 weeks the erythroderma had settled. All three patients also developed aseptic necrosis of either the femoral head or the talus (Fig. 1).

DISCUSSION

The liver metabolises corticosteroids (Goodman & Gilman, 1970), and patients with liver disease are particularly sensitive to therapy with adrenal steroids; they may show the clinical effects of overdosage after quite small amounts (Sherlock, 1963). Furthermore, two of our patients had a low serum albumin and there is evidence that this enhances the peripheral effects of corticosteroids (Lewis *et al.*, 1971). Exogenous glucocorticoids suppress the pituitary–adrenal axis and in the presence of liver disease this suppression is therefore likely to be potentiated. In such cases failure to apply topical corticosteroids may result in an Addisonian crisis as happened in our two patients.

Cirrhosis is known to be associated with aseptic necrosis (Jones, 1965; Jones & Engelman, 1967). The precise mechanism is still uncertain but fat emboli from the liver have been incriminated (Jones & Engelman, 1967). We suggest that in our patients the exogenous steroids were a contributing factor. The effect of steroids may be due to impaired bone remodelling (Fisher & Bickel, 1971) or to the production of an alteration in coagulation leading to sludging and thrombosis in an area of susceptible blood supply (Cosgriff, 1951).

Clearly, liver failure is not essential to the development of these complications but we believe that it may increase the risk. The purpose of this report is to emphasize the possible complications which may follow topical corticosteroid therapy or small doses of oral steroids in the presence of coexistent liver disease.

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British Journal of Dermatology (1975) **92**, 187.

Tissue nickel levels and nickel dermatitis*

I. NICKEL IN HAIR

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Accepted for publication 8 November 1974

SUMMARY

The determination of nickel by instrumental neutron activation analysis is presented. The ^{58}Ni (n, p) ^{58}Co reaction and high-resolution gamma spectroscopy were employed in these measurements. The nickel content of human hair was found to be more dependent upon the sex of the donor than upon other parameters. Mean values for males (1·01 ± 0·23 ppm) and females (4·21 ± 0·54 ppm) agreed with values for the nickel content of hair obtained by other methods in most cases. Nickel sensitivity was not reflected in the nickel contents of hair in the female.

People are constantly being exposed to nickel, and nickel contact dermatitis has become an important problem in everyday life. In a recent study on the epidemiology of contact dermatitis in North America in 1972, nickel was the most common cause of contact dermatitis (Epidemiology, 1973). Workers are affected in many occupations; in countless others the exposure is the result of environmental contamination. In the general population, women have by far the highest incidence of contact allergy to nickel, and environmental exposure is responsible in a preponderance of cases.

Hair is considered as a minor excretory organ, and its nickel content could possibly serve as a measure of nickel exposure and the degree of nickel sensitivity. The objective of this study was to measure and compare the nickel content of hair from nickel-sensitive patients and from non-sensitive subjects.

Although atomic absorption techniques are capable of submicrogram sensitivity for nickel, wet

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* This research was supported in part under Grant HO 00303, National Institute for Occupational Safety and Health, Public Health Service, Department of Health, Education and Welfare.

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chemical manipulations are frequently required. Using neutron activation analysis, it is possible to carry out nickel determinations with entirely instrumental procedures (hence, the possibilities of loss or contamination are minimal). The determination of nickel by neutron activation analysis is accomplished by either the ^{64}Ni (n, γ) ^{65}Ni reaction or the ^{58}Ni (n, p) ^{58}Co reaction. The former reaction requires radiochemical separation of nickel prior to counting the 2·1 MeVβ from ^{65}Ni (Bowen & Gibbons, 1963). The ^{58}Ni (n, p) ^{58}Co reaction can be made highly selective under proper conditions, and it is suitable for the determination of nickel by instrumental procedures.

The ^{58}Ni (n, p) ^{58}Co reaction has a cross section of 90 mb, and the resulting ^{58}Co decays with a 71 day half life emitting a 811 KeV photon (Lyon, 1964; Lederer, Hollander & Perlman, 1967). Many of the (n, γ) reactions can be inhibited by shielding the sample with cadmium during radioactivation. Cadmium foil of 0·5 mm thickness efficiently absorbs neutrons of less than 0·5 eV, and it has little effect on the (n, p) reactions (Moses, 1964). Hence, samples radioactivated under cadmium foil yield less complex gamma spectra. By careful control of the activation time and cooling period, a high degree of selectivity for nickel can be achieved. The major interference in the gamma spectrum is due to the ^{54}Fe (n, p) ^{54}Mn reaction. The 835 KeV photon from ^{54}Mn, however, is readily distinguished from the 811 KeV photon of ^{58}Co by measurement on a high resolution Ge (Li) detector.

A standard nickel solution containing 100 μg Ni/ml was prepared from Matthey 'Specpure' NiO. Nickel standards for each set of samples were prepared by pipetting dilutions of this solution on to pieces of polyethylene sheet, evaporating to dryness under a heat lamp, and heat sealing the sheets with the NiO residues into small lengths of 'lay-flat' polyethylene tubing.

Hair samples were collected from forty-five subjects (24 male, 21 female; 38 non-sensitive, 7 nickel-sensitive) by cutting with stainless steel scissors. (Nickel sensitivity was established by patch testing with 2·5% aqueous nickel sulphate.) The samples were individually washed in ether and then in demineralized water. The ends were trimmed with plastic scissors to remove possible contamination induced in the initial cutting. Weighed hair samples (500 mg–1g) were sealed in individual lengths of polyethylene tubing. Standards (0·5–5 μg Ni) were similarly sealed in polyethylene tubing. Samples and standards were packed into polyethylene tubes (1 in × 3 in) lined with cylinders formed from 0·5 mm cadmium sheet. Discs formed from this sheet covered the tops and bottoms of the cylinders.

Radioactivation was carried out in the 270° vertical tube at the side of the core of the 100 KW Consort Reactor Mark II at the University of London Reactor Centre. At this position, the thermal flux is 1×10^{12} n/cm^2 s, and the fast flux is 2×10^{12} n/cm^2 s. The duration of radioactivation was 20–22 h.

Following radioactivation, the samples and standards were allowed to cool for 14 days. During this time, short-lived species decayed to a point where they contributed little to the radioactivity of the sample.

Counting was carried out on a 30 cm^3 Ge (Li) detector having 5% efficiency and 2·1 KeV resolution coupled to a 8192 channel memory unit. Data were transferred to magnetic tape and evaluated by computer at the University of London. The programme included the determination of the 811 KeV photopeak by the Covell (1954) method. Samples and standards were counted for 4096 s live time using the automatic 12-position sample changer at the University of London Reactor Centre.

Hair samples from the 45 subjects were analyzed for nickel by the procedures outlined above. Histographic presentation of the results (Fig. 1) disclosed two distinct sets: one ranging from 0·75 to 1·50 ppm Ni and the other ranging from 3·00 to 5·25 ppm Ni. Mean values were 1·01 (s.d., 0·23) and 4·21 (s.d., 0·54) respectively. Only the sex of the subject could be correlated with this bimodal distribution: males corresponding to the former and females to the latter. No relationships between age, nickel exposure or nickel sensitivity could be established. The hair samples of the seven nickel sensitive subjects, all of whom were female, had a mean value of 4·53 (s.d., 0·46) ppm Ni while hair

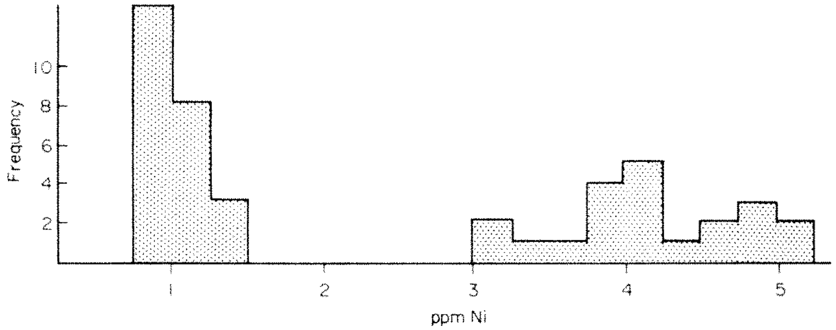

FIGURE 1. Distribution of nickel in human hair.

samples from the non-sensitive female subjects had a mean value of 4·05 (s.d., 0·59) ppm Ni. The Student's *t* and the probability of the difference between the mean values being due to chance are 1·88 and >0·05 respectively. Consequently, there is no significant difference between the mean values, and the nickel content of hair cannot serve as an indicator of the degree of sensitivity.

Significant differences between the nickel contents of hair from the male and that of the female have been reported by Schroeder & Nason (1969). Their analyses were performed by atomic absorption spectrometry, and their sample population was some four times larger than ours. Our mean values tend to be greater than theirs. The difference in nickel content of male and female hair is not as pronounced in the values reported by Eads & Lambdin (1973). They measured the nickel contents of hair samples from ninety-nine subjects by atomic absorption spectrometry and reported higher mean values for males and lower mean values for females than those reported by Schroeder & Nason (1969) and those found by us. Yurachek, Clemena & Harrison (1969) have analyzed human hair by spark source mass spectrometry. They have reported nickel levels for only two samples. While none of these other studies attempted to correlate the nickel contents of hair with nickel sensitivity, they do show mean values in fair agreement with our results. Nechay & Sunderman (1973), however, have reported that there was no significant difference in the nickel content of hair from the male and that from the female. They measured the nickel contents of twenty hair samples (13 male, 7 female) by atomic absorption spectrometry, and they found a mean value of 0·22 ppm Ni (s.d., 0·08 ppm Ni).

Mean values reported by others are compared with our results in Table 1.

From the work of others (Schroeder & Nason, 1969; Eads & Lambdin, 1973; Yurachek *et al.*,

TABLE 1. Nickel levels in human hair according to sex

	Male (ppm)	Female (ppm)
Schroeder & Nason (1969)	0·98 ± 0·15	3·96 ± 1·06
Eads & Lambdin (1973)	1·9	3·4
Yurachek *et al.* (1969)	0·45*	3·4*
Nechay & Sunderman (1973)	0·24 ± 0·009	0·19 ± 0·04
This work		
Nonsensitive subjects	1·01 ± 0·23	4·05 ± 0·59
Nickel-sensitive subjects		4·53 ± 0·46

* Sex of subject not indicated.

1969) as well as from our own measurements, we conclude that there is a significant difference between the nickel content of hair from the human male and that of the female. Furthermore, our work demonstrates that nickel sensitivity is not reflected in the nickel contents of hair in the female.

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British Journal of Dermatology (1975) **93**, 191.

Physiological and environmental control of Gram negative bacteria on skin

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Accepted for publication 7 November 1974

SUMMARY

The relative importance of the environment versus the physiology of the host in the control of Gram negative bacteria on skin has been examined by two experimental designs.

(1) The changes in incidence of Gram negative carriage from three skin sites in a climate controlled chamber at 35°C and 90% humidity for 64 h were determined. Following 2 weeks of tetracycline treatment the above experiment was repeated. High temperatures and humidity increased the overall frequency of isolation of Gram negative bacteria although there were individual differences. This effect was reversed 24 h after a return to the natural environment. Tetracycline inhibited the increase in isolation of Gram negative organisms in six subjects, enhanced it in three and had no effect on one subject. Gram negative populations were less than 10 colony forming units/cm^2 of skin in most instances but ranged up to 10,000/cm^2 skin. No residual effect occurred after return to natural environment and at no time was there a loss of normal flora.

(2) *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were inoculated on normal skin of ten volunteers and survival determined during a 24 h period. Viability was lost between 2 and 6 h on most subjects as compared to 24 h or longer for *K. Pneumoniae* and *P. vulgaris* on an inert surface and between 8 and 24 h for *Ps. aeruginosa*. It was concluded that extremes of environment can affect Gram negative skin carriage but under normal conditions in healthy individuals there is evidence of physiological control of Gram negative flora.

On normal skin in healthy individuals, Gram negative bacteria occur only as transients and in low populations (Marples, 1965). This has been attributed to the degree of moisture on the skin, since these organisms are particularly sensitive to desiccation (Lowbury & Fox, 1953), thus emphasizing the role of the environment as a controlling factor as opposed to the physiology of the host. In an earlier study on the microbial flora of the skin of leukaemia patients (McBride *et al.*, 1974), a high frequency of isolation of Gram negative bacteria occurred in patients who had received neither antibiotic nor antileukaemic therapy of any kind. These results suggested that the disease process may be responsible for changes in the physiology of the skin resulting in an alteration of the biochemical environment, thus permitting survival or a carrier state of Gram negative bacteria. This theory has been postulated for other pathological conditions such as diabetes (Smith, O'Conner & Willis, 1966) although this has not been substantiated (Somerville & Lancaster-Smith, 1973) and in critically-ill patients (Stratford *et al.*, 1968) even though the changes in the skin have not been defined. The use of antibiotics has also been considered as a factor causing a suppression of the normal Gram positive microflora thus permitting the emergence of Gram negatives (Sprunt & Redman, 1968; Leyden *et al.*, 1973).

In view of the increased prevalence of infections caused by Gram negative bacteria, a re-examination of the factors which have been postulated as determinants for the survival of these organisms on skin is timely. Two approaches have been used in this study to establish the relative importance of environment versus the physiology of the host in maintaining a predominantly Gram positive skin flora. First, data collected from a previous study examining the effects of high temperature and humidity environments combined with antibiotics on the skin flora have been reviewed retrospectively to determine whether it is possible to induce a Gram negative carrier state in normal subjects. Secondly, viability of artificially inoculated Gram negative bacteria on normal skin has been determined and compared with that on an inert surface to indicate factors other than desiccation whereby Gram negative bacteria are controlled on the skin surface.

MATERIALS AND METHODS

Experimental design. Ten male volunteers between the ages of 20 and 45 years wearing outdoor clothes were housed in a climate-controlled chamber at 35°C and 90% humidity for 64 h during which time only hand washing was permitted. Four days after their return to the natural environment, the volunteers were treated with 500 mg of tetracycline per day for 2 weeks when they re-entered the climate controlled chamber as described above.

Sampling procedure. Bacterial cultures were taken from the interscapular area of the back, from the groin at a point 2/3 of the distance from the anterior superior iliac spine to the pubic tubercle, and from the soles. Scrubs were taken upon entering the chamber, after 15 h in the experimental conditions and prior to leaving after 64 h. Follow-up cultures were taken from the same areas after 24 and 96 h in the natural environment. Duplicate cultures were taken from the right and left sides of the body by scrubbing 16 cm^2 areas, defined by sterile templates, with calcium alginate swabs moistened in phosphate buffered saline pH 7·2 containing 0·1% Triton × 100. Swabs were returned to 3 ml of collecting medium and shaken for 10 min on a wrist action shaker.

Bacterial techniques. 0·25 ml of each scrub sample was inoculated onto Casman's sheep blood agar and incubated at 31°C for 2 days for identification of the normal flora and Gram negative bacteria. Routine diagnostic techniques were used for identification (Cowan & Steel, 1965).

Survival of artificially inoculated bacteria on skin. Ten male or female volunteers between the ages of 20 and 40 years who did not use antibacterial soaps were used for these experiments. Overnight tryptic soy broth (Difco) cultures of *Proteus vulgaris* ATCC 6386, *Pseudomonas aeruginosa* 14207 and *Klebsiella pneumoniae* ATCC 23357 were centrifuged, washed twice in phosphate buffered saline pH 7·2 and resuspended in 1·0 ml of buffer containing 0·1% Tween 80. Tween 80 was used to lower the surface tension of the solution allowing it to spread evenly on the surface and permit drying within 2 min. 0·05 ml was dropped and spread on delineated areas on the skin of the forearm and air dried. The areas were sampled at the following times: 0, 2, 4, 6, 8 and 24 h, using a modification of the

Pachtman cup scrubbing method with 1 ml of phosphate buffered saline (Shaw *et al.*, 1970). Washings were appropriately diluted, plated on tryptic soy-agar (Difco) using a drop plate method, incubated for 24 h and counted. Gram negative populations were calculated per cm^2 skin and converted to log values. Preparation of cultures for controls was carried out in the same way, but they were inoculated into 13 × 100 mm glass tubes and dried under a stream of air which caused the suspension to disperse and dry as a film over the surface of the tube within 2 min. Films were rehydrated using 1 ml of buffer, shaken in a Vortex junior mixer, diluted and plated as described above.

RESULTS

Changes in the frequency of isolation of Gram negative bacteria with increased temperature and humidity. Table 1 shows the changes in frequency of isolation of Gram negative bacteria on the skin of ten subjects during 64 h at 35°C and 90% humidity. On entering the climate control chamber it can be seen that two of the ten subjects had Gram negative bacteria on their skin and these were on the back. After 15 h at increased temperature and humidity, the frequency of isolation on the back increased to 6 and on the groin and feet to 3 and 2 respectively. After 64 h the frequency of isolation increased slightly, seven out of ten subjects had Gram negatives on their back and four of the ten had them on their feet. The groin showed no response. 24 h after return to the natural environment, the frequency of isolation of Gram negative bacteria declined to near normal values and after 4 days only one subject of the ten harboured Gram negative bacteria on all sites cultured. Thus it can be seen that a moist warm environment causes an increase in the frequency of isolation of Gram negative bacteria on the skin. A return to the natural environment however, very quickly eliminates this effect. The back was the anatomical site where most of the Gram negative bacteria were found and where the effect was most marked. Little change was noted on the feet and the groin, perhaps because these areas are already moist and warm.

TABLE 1. Changes in the frequency of isolation of Gram negative bacteria isolated from skin with increased temperature and humidity

Site cultured	No. subjects/10 with Gram neg. bacteria				
	Time at 35°C, 90% humidity			Natural environment	
	0	15 h	64 h	1 day	4 day
Back	2 (0)*	6 (3)	7 (6)	1 (3)	1 (1)
Feet	0 (1)	3 (2)	4 (1)	2 (1)	0 (0)
Groin	0 (1)	2 (3)	1 (1)	0 (0)	0 (1)

* ( ) after tetracycline treatment.

The frequency of isolation of Gram negative bacteria from skin after these same individuals were treated with tetracycline is also shown in Table 1. The antibiotic appears partially to control the increased frequency of isolation of Gram negatives caused by the increase in temperature and humidity. The incidence was too low, however, to determine the statistical significance. Of particular interest is the fact that there was no residual effect from antibiotic treatment, i.e. return to the natural environment resulted in a decreased incidence of Gram negative bacteria which by the 4th day (post chamber) was at the pre-chamber level. It is also important to note that at no time was there a decrease of normal flora.

Table 2 shows the frequency of isolation of different genera of Gram negative bacteria occurring with increased temperature and humidity. At each culture period, a total of 60 cultures was obtained from the ten subjects. Prior to tetracycline therapy, three species appeared to predominate: *Enterobacter* spp., *Herellea vaginicola* and *Providencia* spp. Following tetracycline therapy, two of these, *Enterobacter* and *Herellea vaginicola* persisted but *Providencia* spp. were mostly eliminated. *Klebsiella pneumoniae* was the only species to show an increased incidence with tetracycline treatment. *Pseudomonas aeruginosa* was isolated only once in 300 cultures indicating a very low incidence in the normal population, as was observed in the earlier study (McBride *et al.*, 1973).

TABLE 2. Gram negative bacteria isolated with increased temperature and humidity and tetracycline

Species	% isolates in 60 cultures											
	Time at 35 °C, 95% humidity						Natural environment					
	0		15 h		64 h		1 day		4 day		Total	
	C*	Te*	C	Te	C	Te	C	Te	C	Te	C	Te
Enterobacter	1·7	0	5	1·7	10	8·3	3·3	5	0	0	20	15
Herellea	0	3·3	6·7	6·7	8·3	5·0	3·3	0	1·7	1·7	20	16·7
Providencia	0	0	6·7	0	1·7	1·7	0	0	1·7	1·7	10	3·4
Mima	1·7	0	1·7	1·7	1·7	1·7	1·7	0	0	0	6·7	3·3
Klebsiella	0	0	1·7	3·3	1·7	3·3	0	1·7	0	1·3	3·3	10
Ps. aeruginosa	0	0	0	0	0	0	1·7	0	0	0	1·7	0
Total	3·3	3·3	21·7	13·3	23·3	20	10	6·7	3·3	3·3		

* C, control untreated; † Te, tetracycline treated.

The results, when viewed collectively, indicate that tetracycline can partially control the increase of Gram negatives with increased temperature and humidity. If the results from each subject are examined individually one can see different effects. Fig. 1 shows changes in the frequency of isolation of Gram negative bacteria from two individuals: Subject 6 shows the expected response to

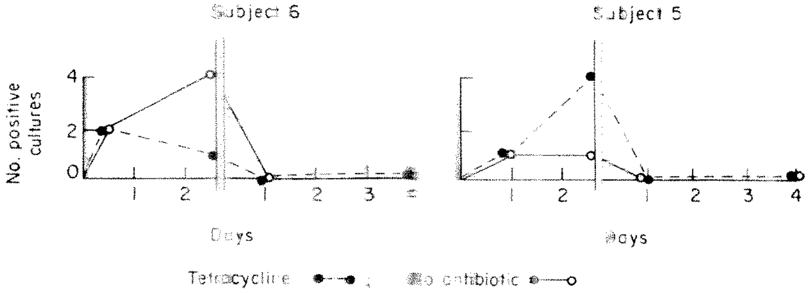


FIGURE 1. Individual changes in numbers of Gram negative isolates with increased temperature, humidity, and tetracycline.

tetracycline treatment; after antibiotic treatment the prevalence of Gram negative isolation fell. The reverse observation is shown with subject 5. Here during the period of the climate control chamber the number of Gram negative species increased after tetracycline treatment as compared with the control period. These two examples are extremes of the response to tetracycline treatment with increased temperature and humidity. Six of the ten subjects studied showed a decline in the number of isolates of Gram negative bacteria after tetracycline treatment, three showed the reverse response, and one subject in the group was completely unresponsive to the antibiotic. It was concluded therefore that while an increase in temperature and humidity can cause an increase in the isolation of Gram

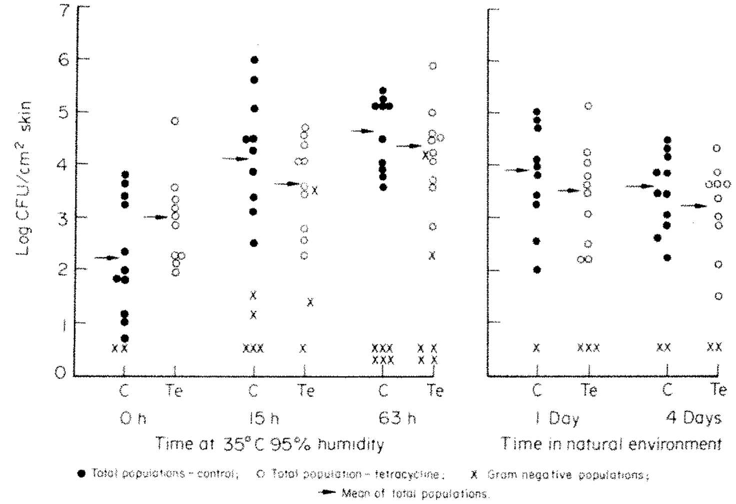


FIGURE 2. A comparison of total bacterial populations and Gram negative populations on the back in a high temperature and humidity environment with and without tetracycline.

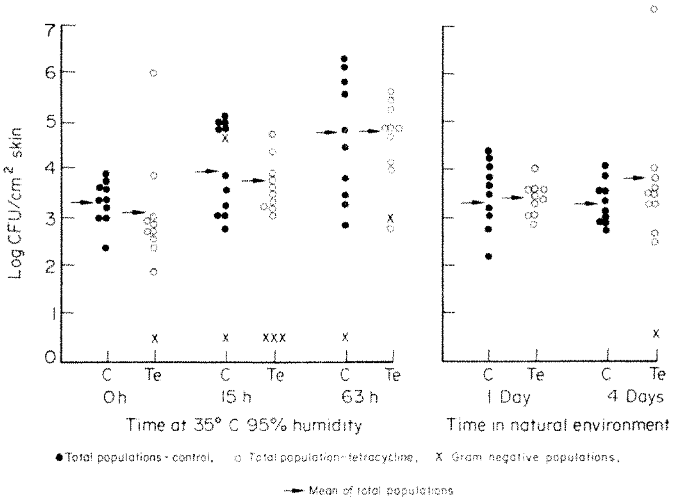


FIGURE 3. A comparison of total bacterial populations and Gram negative populations on the groin in a high temperature and humidity environment with and without tetracycline.

negative bacteria from skin and tetracycline appears to inhibit this activity slightly, in actual fact each individual reacts differently to this form of treatment. It may not be possible to determine prior to antibiotic treatment which subjects may respond adversely.

Comparisons between the total bacterial populations and Gram negative populations from the three anatomical sites studied during this experiment are shown in Figs 2–4. The total bacterial populations increased, with statistical significance, with increased temperature and humidity on the back and groin. The bacterial populations on the feet were greater than on the other two sites and did not react in this way, presumably due to occlusion. Tetracycline treatment did not result in any statistically significant decrease in total populations from the control period. The Gram negative populations were in nearly all instances considerably lower than the total populations and only rarely were greater than 10/cm² of skin. The higher populations were mostly found with increased temperature and humidity. In order to understand the factors responsible for Gram negative population increases it is necessary to follow each subject individually. Table 3 shows the changes in Gram negative populations in the subjects whose populations exceeded 10 organisms per cm² of skin. Again the individuality of the response to increases in temperature and humidity accompanied by tetracycline therapy is apparent. Gram negative populations on the back of Subject 2, for example, were eliminated by tetracycline therapy while the antibiotic had the reverse effect on Subject 4 who was carrying *Klebsiella pneumoniae*.

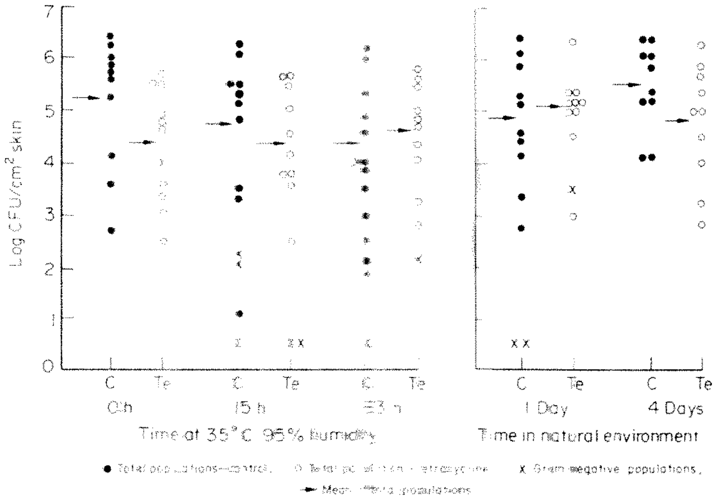


FIGURE 4. A comparison of total bacterial populations and gram negative populations on the feet in a high temperature and humidity environment with and without tetracycline.

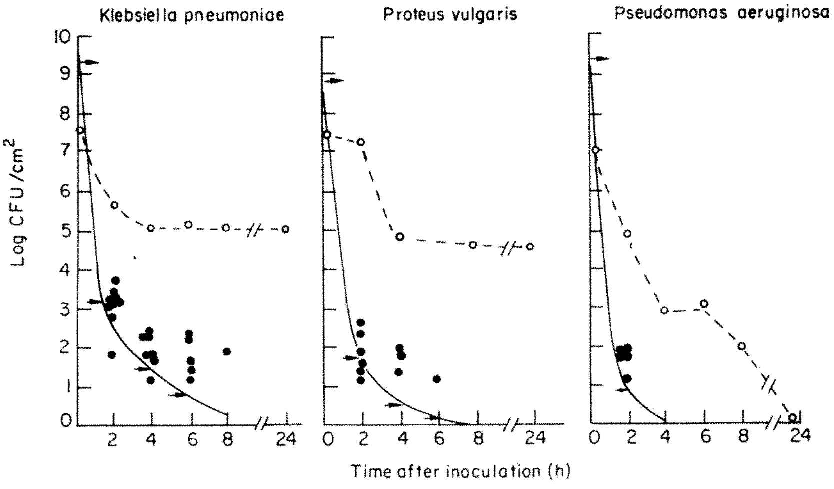
In a number of subjects, high populations of Gram negatives appeared in a random fashion or were higher after 15 h in a high temperature and humidity environment than at 63 h, e.g. the back of subject 5. Similarly there were differences between skin sites, only subject 1 had higher populations on all sites cultured. Hence it would seem that there are other factors affecting Gram negative population levels besides an increase in temperature and humidity.

Survival of Gram negative bacteria on normal skin. Three species—*K. pneumoniae*, *Ps. aeruginosa* and *P. vulgaris*—were chosen for this study because they are rarely isolated from normal skin. The survival of these species on normal skin is shown in Fig. 5 and compared with their disappearance from an inert

TABLE 3. Changes in Gram negative populations at 35°C and 95% humidity with and without tetracycline treatment

Subject no.	Log colony forming units Gram negative bacteria/cm² skin									
	Time at 35°C, 95% humidity						Natural environment			
	0		15 h		63 h		1 day		4 day	
	C*	Te†	C	Te	C	Te	C	Te	C	Te
Back										
1	< 1·0	0	< 1·0	0	0	2·2	0	0	0	0
2	0	0	1·2	0	< 1·0	0	0	0	< 1·0	0
4	0	0	0	3·5	< 1·0	4·2	< 1·0	< 1·0	0	0
5	0	0	3·2	1·4	0	< 1·0	0	0	0	0
7	< 1·0	0	1·5	0	< 1·0	0	0	0	0	< 1·0
Groin										
1	0	0	2·2	0	0	0	0	0	0	0
5	0	0	0	0	4·0	2·0	0	0	0	0
6	0	0	2·1	1·0	2·4	0	0	0	0	0
8	0	0	0	0	1·9	0	0	3·5	0	0
10	0	4·0	0	0	0	0	< 1·0	0	0	0
Feet										
1	0	0	4·8	0	0	0	0	0	0	0
2	0	0	0	0	0	3·0	0	0	0	0

* C, control untreated; † Te, tetracycline treated.



● Individual skin counts; —➤ Mean from 10 subjects; ○ Control–inert surface.

FIGURE 5. Survival of Gram negative bacteria on normal skin.

surface. *K. pneumoniae* proved to be the most resistant to desiccation on the skin. Populations declined rapidly during the first 2 h from the initial log value of 9·28/cm^2 of skin 2 h after inoculation to 3·23/cm^2 (1·9 × 10^8 to 1·6 × 10^3 bacteria/cm^2 of skin). Thereafter the decline was less rapid, but at the end of 8 h only one subject was found to be carrying *K. pneumoniae* and at 24 h this organism was not isolated from any of the subjects. By comparison, bacteria inoculated on an inert surface maintained viability up to 24 h in considerably higher populations. Survival of *P. vulgaris* was comparable to that of *K. pneumoniae* with the exception that the decline in populations on skin was even more rapid. At the end of 4 h only three of the ten subjects had the organisms on their skin and only one after 6 h. *Ps. aeruginosa* proved to be the most susceptible of all the species tested. Two hours after inoculation only five subjects were found to carry the organism and none were carriers after 4 h. *Ps. aeruginosa* on an inert surface also proved to be more susceptible to desiccation and survived in lower populations and for a shorter period of time than the other two species tested. There was a continual loss in viability during the 8 h sampling period with no recovery at 24 h unlike *P. vulgaris* and *K. pneumoniae*. Individual differences were noted in self disinfecting activity of the skin as shown in Table 4, where the time interval for loss of Gram negatives can be seen to vary as much as 6 h between subjects.

TABLE 4. Survival times of artificially inoculated Gram negative bacteria on skin

Species	No. subjects/10 with Gram negatives				
	Time after inoculation				
	0	2 h	4 h	6 h	8 h
K. pneumoniae	10	10	8	6	1
P. vulgaris	10	8	3	1	0
Ps. aeruginosa	10	5	0	0	0

DISCUSSION

The control of the microbial flora of the skin elicited a great deal of interest and speculation among the early workers in the field of skin microbiology. In a thorough review of the early work Burtenshaw (1948) concluded that evidence exists for the presence of a self-disinfecting activity of the skin with the following contributing factors: desiccation, acidity, fatty acids and 'other ill-defined agents'. Since that time the effect of acidity as an antibacterial agent has not been substantiated because most skin bacteria can proliferate over a wide pH range (Pillsbury & Rebell, 1952). The role of skin lipids in the control of Gram positive populations first described by Burtenshaw (1942) has been substantiated by others (Ricketts, Squire & Topley, 1951; Aly *et al.*, 1972), but has little effect on Gram negatives. Desiccation has been postulated as the major factor in the control of these species. Studies by Rebell *et al.* (1950) on a comparison of the survival of gram negative species on skin and on glass surfaces showed survival times of less than 20 min with no differences between the two surfaces. The discrepancies between their results and those we have reported here may be attributed to the fact that desoxycholate media were used for the recovery of the organisms. In a preliminary study on plating media for these experiments we found commercially prepared desoxycholate media inhibitory to Gram negative bacteria after they had been dried.

The results recorded by Petit & Lowbury (1968) are in accordance with our findings. While only a small percentage of the initial inoculation survives drying, the remaining bacteria are resistant to this effect and survive. Many factors may contribute to the discrepancies between experimental results, these include variability between strains which has been demonstrated by Lowbury & Fox (1953), inoculation size and methods of recovery. While there are considerable differences in populations after drying, the important finding is the differences in the length of time of survival between skin and an inert surface. The rapid disappearance from skin must be attributed to some intrinsic character of normal skin.

The results presented here point to the fact that while extremes of environment can cause temporary changes in skin carriage of Gram negative bacteria, control of skin flora has a predominately physiological basis, the factors for which have yet to be identified.

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Pruritus vulvae: treatment by multiple intradermal alcohol injections

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Accepted for publication 1 November 1974

SUMMARY

A preliminary report is presented on the use of multiple intradermal injections of absolute alcohol in the treatment of intractable pruritus vulvae. Twenty-five patients were followed up for over 1 year, and of these six (24%) were cured, and thirteen (52%) showed marked symptomatic improvement.

Pruritus caused by benign lesions of the vulval epithelium is commonly a difficult and persistent problem for the gynaecologist. Often a vicious cycle of symptoms develops. The more a woman scratches, the more she itches, the vulval skin becoming progressively more traumatized and inflamed.

In an effort to interrupt this cycle, we have treated over thirty patients by the method described by Woodruff & Thompson (1972) using multiple intradermal injections of absolute alcohol.

Twenty-five of these patients have been followed up for over a year.

PATIENTS AND METHOD

Patients treated were aged 30 to 77 years (mean age 59·8 years); all but two were postmenopausal. Pruritus was the main and often the only symptom although two patients also suffered from dyspareunia. No patient with pain as her primary symptom was included in this series. Women were only selected for study if their symptoms had been present for over a year and had not responded to standard therapeutic measures, including topical steroid applications, psychotropic therapy, and in nine cases simple vulvectomy.

Local infectious processes such as trichomoniasis and candidiasis were excluded in all cases, and in careful history-taking and general examination search was made for causes such as systemic disease, other skin diseases, the application of local irritants (Ward & Sutherst, 1973) and the use of unsuitable underclothing (McFarlane, 1973; Strunin, 1973).

Vulval biopsy was performed in all patients prior to treatment. Histological examination showed lichen sclerosus et atrophicus (LSA) in thirteen cases, chronic dermatitis with a varying degree of hyperkeratosis in eleven cases, and in one case normal vulval skin (Table 1).

Under general anaesthesia the ano-genital area was marked out with ink in one centimetre squares

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TABLE 1. Symptomatic response of patients to treatment

Case no.	Age	Duration of symptoms (years)	Histology	Results of alcohol treatment
1	59	3	L.S.A.	Cured
2	55	13	L.S.A.	Marked improvement
3	71	2*	Chronic dermatitis	Cured
4	65	8*	Chronic dermatitis	Marked improvement
5	40	1	L.S.A.	Marked improvement
6	52	4*	Chronic dermatitis	No improvement
7	64	16*	L.S.A.	Marked improvement
8	74	2	L.S.A.	Marked improvement
9	44	8	L.S.A.	Marked improvement
10	77	3	Chronic dermatitis	No improvement
11	30	1	Chronic dermatitis	Slight improvement
12	70	1*	Chronic dermatitis	Cured
13	58	1	L.S.A.	Slight improvement
14	57	6*	L.S.A.	Cured
15	58	10*	L.S.A.	Marked improvement
16	60	3	L.S.A.	Marked improvement
17	55	10	Chronic dermatitis	Marked improvement
18	64	10	Chronic dermatitis	Cured
19	67	2	Normal histology	No improvement
20	64	13	L.S.A.	Marked improvement
21	71	1	Chronic dermatitis	Marked improvement
22	52	5*	Chronic dermatitis	Slight improvement
23	57	15	Chronic dermatitis	Cured
24	67	10	L.S.A.	Marked improvement
25	63	4*	L.S.A.	Marked improvement

* Indicates previous vulvectomy.

(Fig. 1). At each line intersection 0·1–0·2 ml of absolute alcohol was injected intradermally. A fine 25 gauge 15 mm needle was used, the point being directed to the deeper part of the dermis (i.e. about 5–6 mm below the skin surface). Alcohol is well known for its ability to cause skin necrosis, and care was taken to inject neither too superficially, which may produce small blisters, nor too deeply, which may cause areas of cellulitis or focal sloughing (Woodruff & Thompson, 1972). However, these complications can be avoided by directing careful attention to technique.

Most patients developed some degree of post-operative discomfort, but this was relieved by mild analgesics in all cases. Each patient was able to leave hospital on the 1st or 2nd post-operative day. All patients experienced local swelling for 2–3 weeks after therapy, and skin sensation was lost for up to 2 months. Explanation of the operation to the patient allayed any anxiety these side effects might have caused.

RESULTS

Table 1 shows the response of patients to treatment. Of the twenty-five patients, six (24%) were cured and required no further treatment at all. Thirteen patients (52%) showed marked improvement. These needed only very occasional topical steroid applications to keep them symptom free. Three

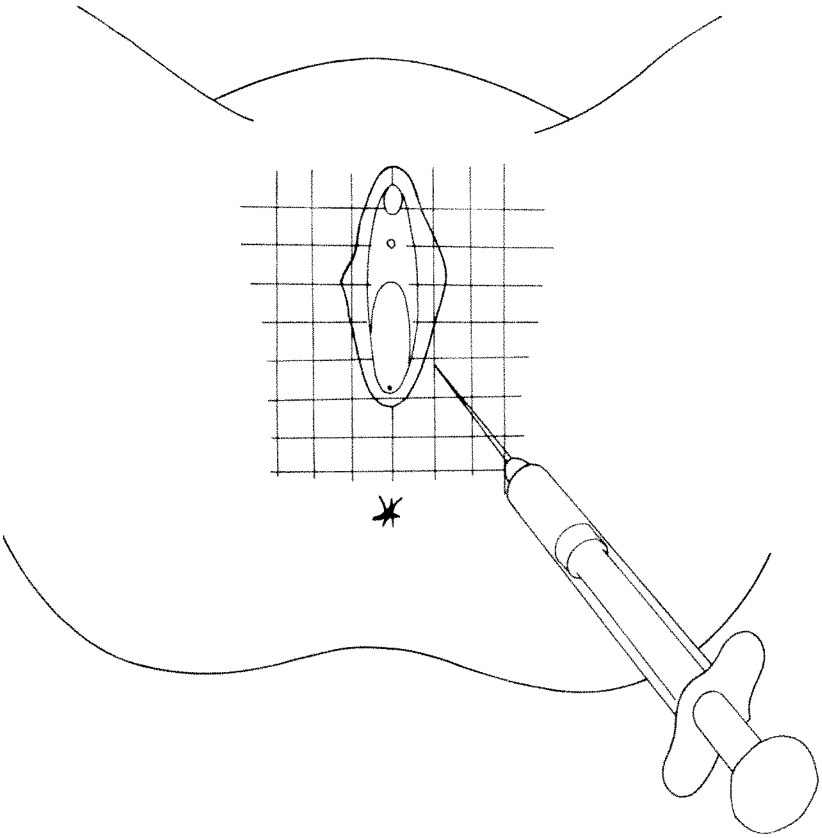

FIGURE 1. The vulva is marked out with ink in 1 cm squares. Injections are made at each line intersection.

patients (12%) said they were better but still had some pruritus, and applied topical steroids regularly. In three patients (12%) treatment with alcohol injections had no effect. Two of these were badly controlled diabetics, and it was felt that this was largely responsible for their poor response. Two patients developed superficial skin blistering, and one developed mild cellulitis which resolved spontaneously.

DISCUSSION

Many methods are employed in the treatment of pruritus vulvae, but sometimes fail. Topical application of local anaesthetics, steroids, oestrogen and testosterone commonly provide only temporary relief. There is often a psychological element in the aetiology, but psychiatric assessment and interview, and therapy with psychotropic drugs do not always provide much benefit.

The prolonged dampness associated with modern female underwear, antiseptics and detergents added to bath water, and vaginal deodorants and anti-perspirants, all aggravate the condition (McFarlane, 1973; Strunin, 1973; Ward & Sutherst, 1973).

When all else fails recourse is often taken to surgery, but even the ablative procedure of vulvectomy does not necessarily cure the condition (nine of this series of patients had had simple vulvectomy performed previously).

The denervation operation described by Mering (1952), is an alternative surgical approach, but again may be regarded as a major procedure, and we believe a symptom such as pruritus ought to be amenable to more simple treatment.

Intradermal injection of alcohol offers a form of therapy intermediate between local medication and major surgery; its aim is to provide temporary loss of sensation to the affected area and thereby break the cycle of symptoms. When skin sensation does return it does so without pruritus because the vicious circle has been broken.

Complications of the treatment are few, are easily treated, and time in hospital is short.

We appreciate that a controlled double-blind trial on this method of treatment is necessary, and this is currently being undertaken.

ACKNOWLEDGMENTS

We wish to thank Mr D.H. Lees and Mr T. Smith of the Jessop Hospital, Sheffield for allowing us to treat their patients.

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British Journal of Dermatology (1975) **93**, 205.

Pharmacology and Treatment

Simplified dithranol treatment for psoriasis*

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Accepted for publication 9 December 1974

SUMMARY

Progressive simplification of dithranol therapy has made treatment at home more acceptable. This has included omitting ultra-violet light, talc and dressings, and substituting an ordinary bath instead of one containing tar. A stiffened dithranol 0.5% ointment was found to be slightly more effective than the best paste hitherto employed. It was also very much easier to use.

In spite of the staining it produces, dithranol has been the mainstay in the physical treatment of psoriasis in Lancaster since 1953. This has been on an in-patient basis for geographical reasons and so that patients can be more readily observed and trained by available staff. Despite concentrating so much on one form of therapy it had not been possible to persuade many patients to restart their treatment at home when they had been unfortunate enough to relapse. It was observed that the inconvenience of the 'tar' bath and ultra-violet light was often used as an excuse for abandoning treatment; in addition there was difficulty in obtaining and using paste, powder and dressings and the time that was involved. This paper describes how the regime has been progressively simplified in an attempt to make home treatment possible and more acceptable.

MATERIALS AND METHODS

183 consecutive non-pustular non-exfoliative in-patients were studied personally over a 3-year-period from 1968. Pastes were made from the same batches of ingredients. Only trained staff were employed, and patients were allocated so that one person did most of the treatment. Frequent checks were made to see that the treatment was kept constant; complete clearance was the only criterion for cessation of therapy throughout. Assessment by a concurrent trial was rejected, as it would be impossible to exclude staff and patient bias, and could not be carried out on a 'blind' basis because of the materials used. The following method was therefore adopted: halfway through the assessment the ultra-violet light was stopped and the tar omitted from the bath. Whilst being prepared for a longer initial treatment time, it was thought that patients would realize it was the dithranol that was effective and would use it for relapses. In any event there would be considerable saving in treatment effort, time and expense. The results were unexpected, and more than justified the progressive simplification.

Experiment 1. (Omission of ultra-violet light and tar bath)

The paste had been standardized after demonstrating by double blind experiments that the addition of 15% hard paraffin gave the best results, the dithranol being used at 0·5% for most patients (Seville, 1966). The response then depends on the number of applications per week, and it was found on average that there were 1·5 rest days. A mean figure of 19 treatment days followed the use of sunlight and tar baths, but without them it was 16·5 days; this result is not significant ($P > 0{\cdot}2$). MacLennan & Hellier's (1961) treatment time was 15·2 days based on a 5-day week. It is interesting that it was only possible to approach this figure when the sunlight and tar baths were omitted. The current series was therefore broken down further. Patients were paired on the basis of age, sex, extent and activity of the eruption, and number and type of admissions. As is shown in Fig. 1, there is still the same tendency for slight reduction without ultra-violet light and tar baths, but again it is not statistically significant. A 3-year follow-up was then carried out to see if there was any difference in the rate of relapse between the two treatments; there was a very slight tendency for patients treated with sunlight and tar baths to relapse more quickly.

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* Based on papers given to the Dowling Club in Lancaster and Holland 1973/4.

UVL and tar baths	With	Without	P
First admissions	25·1 ± 12	21·9 ± 12·1	>0·3
	68 admissions compared		
Relapses	29·9 ± 10·5	27·1 ± 13	>0·2
	20 admissions compared		

Time in days

FIGURE 1. Time for clearing of psoriasis using dithranol with or without u.v. light and tar baths.

Comment. It is not possible to explain why there is a tendency for the patients who were not treated with sunlight and tar baths to clear more quickly and to remain so for slightly longer periods. The use of stiff 0.5% dithranol from the onset was introduced in 1960; it is very doubtful if there was any improvement in the method during the 3 years from 1966 in view of the precautions already described.

These findings certainly confirm those of Young (1970) who, using the different method of direct comparison, was not able to demonstrate any advantage from ultra-violet light and the tar bath.

Experiment II. (*Improving the base for the dithranol*)

From the experience with pastes it was thought that any base for the dithranol that could be used more easily at home would still have to be stiff to be effective. Equal quantities of hard and soft paraffin B.P. were prepared with 2·5% chloroform as a dispersant for the 0·5% dithranol. Salicylic acid 2% was added for this assessment as it is present in Lassar's paste. As one of the preparations was a stiff ointment and the other a stiff paste, it was not possible to have a complete double blind comparison, but the method was otherwise as previously described (Comaish, Smith & Seville, 1971). Seventy-two patients were assessed; twenty responded equally, the paste was better in twenty-two but in thirty the ointment was better.

Comment. The slight tendency for the ointment to be better ($P=0{\cdot}25$) is surprising as it is not as stiff as the paste. Its advantages were that it was much easier and quicker to apply, there was no need to break down the cohesion by hand, much less was required, and it did not need removing with oil before bathing. Milling was not necessary, and it was much easier to prepare. Following the above results the ointment was prescribed for thirteen consecutive out-patients previously treated with paste at home. All preferred it, found it more effective, and commented that it did not drop on to and ruin the bedroom carpets. The most important result was that they were actually doing their treatment.

Experiment III. (The role of dressings and powder)
All the thirteen out-patients just mentioned had adopted the suggestion of treatment at night-time, followed by wearing old pyjamas; only one applied dressings and two used powder. The need for dressings and powder was therefore assessed on eight consecutive in-patients, but the advantage was obvious in all.

Comment. The use of dressings and powder has been retained for in-patients to date, and reserved for out-patients who spread their ointment while scratching. Incidentally Young (1970) and Harris (1972) have discontinued them, but those workers were using pastes.

Details of the dithranol ointment
Salicylic acid is needed in the prescription as was shown by a double blind trial, comparing a 9-month-old ointment containing 2% and a fresh batch made without it. The first four consecutive patients responded better to the fresh ointment. By then 6 weeks had elapsed, and the remaining twelve patients with one exception responded better on the salicylic acid side. The last-mentioned result is significant ($P = 0{\cdot}006$) but applies only to part of the trial. Determining the optimum strength with regard to protection of the dithranol and clinical efficacy is the subject of work to be submitted (Bellis, Seville & Smith, 1975). Salicylic acid at 0.5% strength seems satisfactory, as an ointment stored on hot pipes for 4 months, a destructive procedure for pastes, was as effective as fresh material in twelve patients. This strength has the advantage that it can be readily dispersed with the dithranol in chloroform as follows:

Dithranol	0·5%
Salicylic acid	0·5%
Chloroform	2·5%
Hard paraffin B.P.	
White soft paraffin B.P. equal quantities to 100%	
(Suggested dispensing size 100g)	

Note for Pharmacist: Heat paraffins until just melted. Triturate until smooth and brilliant white, 38°C. Add chloroform mixture. Triturate until cool. Rub down on slab until smooth. Store in dark.

It is advisable to use a hard paraffin of low melting point in the B.P. range such as 53/55°C to prevent crystallisation; the soft paraffin has a melting point of about 49°C.

Comment. The preparation would appear to have a shelf life to date of more than 1 year. This would therefore compare very favourably with the short shelf life commented on by Ponec-Waelsch & Hulsebosch (1974) with dithranol zinc oxide pastes containing salicylic acid up to 0·6%.

DISCUSSION

Farber & Harris (1970) and others have advocated using weaker concentrations of dithranol such as 0·1% or 0·2%. This presumes a daily treatment with increasing strengths of dithranol as necessary. It has been found, however, that undertaking most of the treatment with the 0·5% preparation has simplified management and reduced treatment time. It has made it easier for patients to obtain supplies through local chemists and to be more confident whilst treating themselves; not having to use local therapy every day of the week is certainly appreciated.

Teaching the therapy is also easier. The patients are instructed to use the 0·5% dithranol sparingly on thicker areas first until the reaction can be judged, and to control it by the quantity used and the number of applications per week. The only severe reactions with this strength have occurred in patients previously treated with potent local steroids. Patients are therefore given written instructions not to use this strength for at least a month after the last application of potent steroid preparations, and then with extreme caution for half a year. They are also instructed to stop treatment for a week if there is moistness, cracking and/or soreness, or if the psoriasis spreads while producing any of these symptoms. No limit has been placed on quantities used as there have been no systemic toxic effects to date; nor have any followed the prolonged and extensive use of dithranol 0·5% paste, a finding confirmed by other workers (Ippen, 1959; Champion, 1966; Farber & Harris, 1970; Harris, 1972). Nursing staff and patients accustomed to weaker strengths should be cautious when starting with stiff 0·5% ointment and apply it sparingly, because it is initially much quicker in action than the paste; patients are sometimes too enthusiastic in the use of the ointment as it is so much easier to apply. Many of the details of this paper are contained in a treatment proforma for practitioners of which the patient is given a copy, with opportunity for discussion at the beginning of the course. Particular emphasis is made in it of continuing treatment until the skin is entirely clear, that is, when there is nothing to feel with the fingers and the texture is normal and the dithranol stain is then seen peeling off leaving skin of normal colour.

ACKNOWLEDGMENTS

I would like to acknowledge the team work from Mr Grantham and Nursing Staff, to Miss Dorothy Till for the typing, Mrs Shirley Barber for the figures, Mr Trevor Walker for the statistics, and to Professor M.W. Greaves, Drs D.B.Brookes, S. Comaish and G.B. Walker, for comments and help with assembling the material.

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British Journal of Dermatology (1975) **93**, 209.

The absorption of hexachlorophane from Ultralanum ointment

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Accepted for publication 4 November 1974

SUMMARY

The application of Ultralanum ointment to adult skin cases has produced levels of hexachlorophane in the plasma comparable with those found in infants who have undergone whole-body washing with hexachlorophane solutions. Clinical evidence of toxicity was not observed, but it is felt that the value of hexachlorophane in topical steroid preparations should be re-appraised, particularly for long-term treatment of large eczematous areas.

The incorporation of a bacteriostatic or bactericidal agent into topical steroid preparations is desirable to control secondary infection promoted by the steroid. Many such agents have been used —hexachlorophane, clioquinol, neomycin, urea and F.A.P.G. base, to mention a few—and all have advantages and disadvantages. Recently the safety of hexachlorophane, when used as a topical agent, has been called into question.

That the inappropriate use of topical hexachlorophane can produce neurotoxic effects is clearly established. This can occur when the concentration is too high, as in the faulty dusting powder containing 6% of hexachlorophane that caused the deaths of several infants in France, and when the barrier to penetration is abnormally weak, as when four women treated with 3% hexachlorophane vaginal packs retained for several days were so severely affected that two died (Food and Drug Administration); and when the repeated washing of extensive burns with hexachlorophane resulted in neurotoxic symptoms and six deaths (Food and Drug Administration).

However, despite the frequently-repeated exposure of surgical teams to hexachlorophane solutions, and even fairly frequent whole-body exposure to hexachlorophane-containing soaps, toxic effects do not seem to result from the application of hexachlorophane in approved concentration to the normal skin (Pittman, 1972; Gluck, 1971; Kennedy, 1971). Infants whose epidermis is thin and whose surface:volume ratio is high must be presumed to be at greatest risk, and it is true that post-mortem reports on sixty-nine infants, who had died of unrelated causes during the perinatal period in a hospital where routine daily washing with 3% hexachlorophane was employed, showed that seven had spongy changes in the C.N.S. myelin. However, in addition to being 8 weeks premature and weighing

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no more than 1400 g, all of the affected infants also had skin rashes, abrasions or wounds (Powell *et al.*, 1973).

Since the absorption of medicaments through intact but diseased skin can be abnormally high, it is important to know whether the apparently safe levels of hexachlorophane resulting from the use of hexachlorophane-containing soaps and cleansing agents on normal skin are exceeded when a corticosteroid ointment containing hexachlorophane is applied to diseased skin.

MATERIALS AND METHODS

Ultralanum ointment, containing 1·4% of hexachlorophane, was applied to six adult patients and the plasma hexachlorophane was measured after various periods of treatment according to ordinary clinical practice.

The extraction of hexachlorophane (HCP) and its estimation by gas chromatography were carried out by the methods of Porcaro, Shubiak & Manowitz (1969); Browning *et al.* (1968); and Curley & Hawk (1971).

To 0·2 ml plasma were added 0·2 μg bromchlorophene (BCP), 0·8 ml water, and 20 μl concentrated hydrochloric acid. After ultrasonic extraction for 3 min with 6 ml of a mixture of ether and chloroform (3:1 by volume), the organic phase was separated and the solvent evaporated. The residue was removed with 0·5 ml methyl alcohol, 4 ml ether and 0·5 ml of a 1-molar solution of diazomethane in ether. After 5 min the mixture of reagents was dried and the residue was dissolved in 1 ml N hexane.

Part of this solution was analysed by gas chromatography. The requirements for the separation were: 2 m glass column, filled with 1% OV 17 on Chromosorb G-HP; column temperature, 235°C; gas vehicle, N_2, 20 ml/min; detector, ^{63}Ni-EC. The ratios of the peak levels of HCP and BCP were calculated for the quantitative evaluation, and were compared with the corresponding values of solutions containing BCP and HCP in known concentrations (1 μg/ml BCP and 0·25, 0·50, 0·75 and 1·00 μg HCP/ml respectively). The method is sensitive to 0·01 μg/ml.

RESULTS

Table 1 shows the plasma levels of hexachlorophane found in our cases.

DISCUSSION

Unfortunately no threshold level for the production of neurotoxic symptoms has been established in humans, owing to the extreme paucity of information about the peak levels reached in patients who have experienced toxic effects. The only patients in whom plasma levels of hexachlorophane were measured at the onset of neurotoxic manifestations seem to be three adults with extensive burns undergoing daily washing with 3% hexachlorophane (Larson, 1968). One, whose plasma level fluctuated daily between 12·3 and 14·0 μg/ml before washing and 28·6 and 29·2 μg/ml after washing, complained of diplopia. Another, who experienced vomiting and extreme irritability, had a plasma level of 35·0 μg/ml; while at a level of 74 μg/ml the third patient had convulsive seizures. (All these neurological symptoms cleared completely within 3 days and did not recur.) Yet another patient experienced no neurological symptoms with a plasma level of 34 μg/ml, which remained above 12·8 μg/ml for 5 days after stopping treatment.

It has been suggested, however, that the laboratory technique used in Larson's cases may not be comparable with more recent methods for measuring hexachlorophane in the blood (Lockhart, 1972). In only two other cases of evident intoxication, both of whom died, do plasma levels seem to have been

TABLE 1. HCP values (μg/ml plasma) in patients with skin diseases, after cutaneous application of Ultralanum ointment (St Helier Hospital)

Patient	Sex	Age	Reason for treatment	Amount of Ultralanum ointment prescribed (g per week)	Area under treatment	Duration of treatment with ultralanum ointment	Equivalent hexachlorophane μg/ml plasma
D.C.	M	75	Pruritus	25	All areas of body	15 months	0·03
G.P.	F	65	Eczema	60	Trunk	2 weeks	0·29
J.F.	M	60	Eczema	50	Arms, legs	4 weeks	0·08
R.D.	M	50	Eczema	15	Hands	17 months	0·13
E.R.	F	76	Seborrhoeic dermatitis	40	Trunk	3 weeks	0·04
W.S.	M	69	Eczema	50	All areas of body	4 months	0·30

measured, but this was some time after hexachlorophane had been withdrawn. Although after an uncertain interval of probably 24–48 h a plasma level of only 4·4 μg/ml was found, we cannot conclude that this level, which is much below the levels found in Larson's affected patients, is above or below the threshold for neurotoxicity.

The threshold plasma level for changes in the myelin of the rat's central nervous system is below this level, since Curley & Hawk (1972) produced such changes by maintaining plasma levels between 1·97 and 2·96 μg/ml. At levels up to 0·65 μg/ml no effects on the brain were seen (Curley & Hawk, 1972). The threshold for clinical toxicity in humans probably lies above 2 μg/ml, since Gluck (1971) found a maximum value slightly above that level in a group of nine unaffected adult volunteers who exposed their whole body surface to a 3% hexachlorophane soap for 5 min twice daily for 60 days. The mean plasma level was 1·36 μg/ml (Gluck, 1971). Kennedy, who used ten adult subjects, noticed no toxic effects from 10 min heavy lathering with a 0·75% hexachlorophane soap daily for 21 days. He found an equivalent plasma level of 1·46 μg/ml with a mean level of 1·04 μg/ml in males and 0·56 in females (Kennedy, 1971).

Schering AG. Berlin found that the daily inunction of 10 g Ultralanum ointment for 7 days to normal skin of the upper arm of ten healthy volunteers produced a maximum plasma level of 0·28 μg/ml with a mean of 0·14. In a series of seven patients treated with Ultralanum ointment and studied by Schering the maximum plasma level reached was 0·29 μg/ml except for the single case of infantile eczema, in which the highest level recorded was 1·01 μg/ml.

The results in the adult cases are similar to our own. It is of interest that there is a tenfold difference in concentration between the patient with pruritus who had been treated for 15 months to all areas of the body, and the patient with eczema of the trunk who had been treated for 2 weeks.

These results, although too few for firm conclusions to be drawn, suggest that the application of a hexachlorophane-containing ointment to extensive areas of eczematous skin can, even in the adult, produce plasma levels of hexachlorophane which are comparable with the levels that can be produced by whole-body washing of the intact skin in infants. There was no obvious clinical evidence of toxicity in our patients but subclinical toxicity cannot be excluded. A more detailed assessment of neurological function, including E.E.G.s, would be valuable in any further studies.

It is felt that the value of hexachlorophane in topical steroid preparations should be re-appraised in the light of our findings, particularly when long-term therapy of large eczematous areas is necessary.

TABLE 2. HCP values (μg/ml plasma) in patients with skin diseases after cutaneous application of Ultralanum ointment (Schering AG)

Patient	Diagnosis	Treatment and blood sample	Days 1	2	3	4	5	6	7	8	9	10	11	12
		Ultralanum ointment												
		2 × 5 g	−	−	−	+	+	+	+	+	+	−	−	−
		Blood sample 18.00 hrs	+	−	+	−	−	+	−	−	+	−	−	+
R.R.	Impetigo contagiosa with infantile eczema		<0·01		<0·01			0·22			1·01			0·22
R.F.	Scabies with marked eczema		<0·01		<0·01			0·24			0·29			0·21
		Ultralanum ointment												
		2 × 10 g	−	−	+	+	+	+	+	+	+	−	−	−
		Blood sample 08.00 hrs	+	+	−	−	−	−	+	−	+	−	−	+
H.W.	Generalized psoriasis vulgaris		<0·01	0·02					0·19		0·12			0·14
U.M.	Generalized endogenous eczema		0·05	0·02					0·27		0·15			0·20
M.S.	Seborrhoeic senile erythroderma		<0·01	<0·01					0·10		0·11			0·08
H.S.	Generalized chronic eczema + pulmonary tuberculosis		<0·01	0·04					0·16		0·21			0·22
G.U.	Generalized seborrhoeic eczema		0·03	0·03					0·08		0·09			0·08

ACKNOWLEDGMENT

We thank Dr K.Luebke of Schering AG Berlin for carrying out the estimations of hexachlorophane and for supplying the results of Schering's own investigations.

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British Journal of Dermatology (1975) **93**, 215.

Case Reports

Generalized bullous and haemorrhagic lichen sclerosus et atrophicus*

MARKED IMPROVEMENT WITH ACTH

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Accepted for publication 4 November 1974

SUMMARY

An unusual case of lichen sclerosus et atrophicus is described, in which lesions were bullous and haemorrhagic and extended over almost the entire surface of the skin. A marked improvement followed treatment with ACTH.

CASE REPORT

The patient, a male aged 64 years, with no relevant past or family history, started to develop lesions on his arms, neck, flanks, clavicular region and back, at the age of 57 years (Fig. 1).

In some areas the lesions were rounded and discrete, and in others they overlapped to produce extensive areas with irregular edges. Initially, the skin was only erythematous but later it became increasingly thinner, parchmentlike and finally whitish. These changes spread to involve the inguinal folds and thighs, and especially in these regions some lesions developed purpuric festooned bullous areas at the edge of the atrophic patches. Later these developed into small superficial ulcers.

Investigations

The following investigations were performed: blood glucose, 140 mg%; blood urea, 26 mg%; haemoglobin, 16 g%; E S R 4 mm/1h; leukocytes, 7·500/cmm; serum proteins, 7·1 g% (albumin 55%, α_1-globulin 4%, α_2-globulin 9%, β-globulin 14%, γ-globulin 18%); rheumatoid factor and LE cell tests, negative.

Antibodies directed against nuclear antigens (ANF) or basement zone of stratified epithelia were not demonstrated. Direct immunofluorescence demonstrated the presence of immunoglobulin deposits (IgG, IgM, IgA), complement (C3, C4) and fibrinogen in the colloid bodies of the damaged skin and in clinically normal skin.

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* This work has been partially supported by C.N.R.—grant. no. 72.01141.65.

Histology

This showed hyperkeratosis with follicular plugging. The stratum Malpighii was oedematous and thinned, and the basal cells showed hydropic degeneration. The dermal papillae were flattened. The sub-epidermal zone showed oedema, and the connective tissue, which stained poorly with usual stains, showed homogenization of the collagenous fibres and few cells; it appeared to be lymphoedematous rather than sclerotic. Only a small number of inflammatory cells—lymphocytes and histiocytes—were observed in the mid-dermis, and the adnexal structures were reduced.

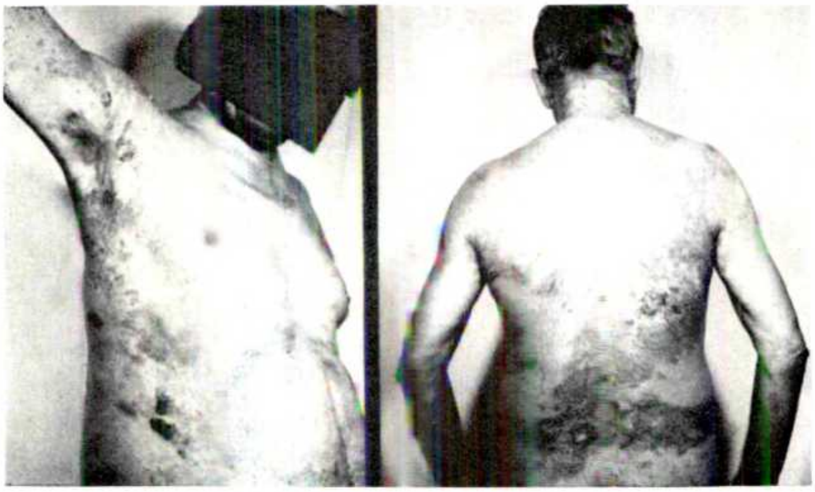

FIGURE 1. Almost the entire skin surface shows whitish, parchment-like and sclero–atrophic lesions. Along the edges there are purpuric haemorrhagic festooned patches with superficial ulceration.

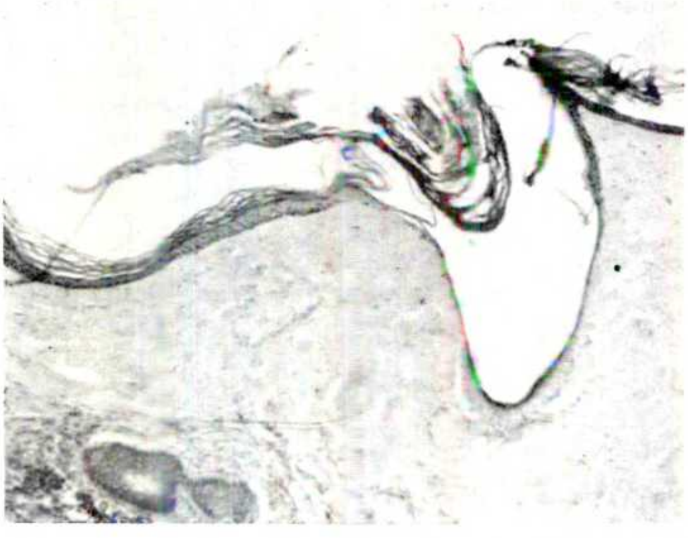

FIGURE 2. Histopathology showing changes of lichen sclerosus, including bulla formation and extravasation of red cells.

Treatment and progress

During his latest hospital admission the patient got worse despite therapy with antiseptic ointments, and 0·5% metandrostenolone cream topically and vitamin A, anti-inflammatory drugs and tranquillizers systemically. He was, therefore, given ACTH 1 mg on alternate days for eight doses. Two weeks later there was marked improvement and no new lesions. Three months later he has no symptoms and is free of active lesions.

DISCUSSION

Lichen sclerosus et atrophicus is a chronic disease first described clinically by Hallopeau (1889, 1896) and histologically by Darier (1892). It can occur at any age but is more prevalent in adult women around the menopause than in men. It most often attacks the ano-genital area. The unusual features of this case were the sex of the patient, the presence of haemorrhagic areas and bullae at the edges of the lesions, the extent of the eruption, and the response to ACTH treatment. The characteristic histological changes in the subepidermal region do not make the appearance of bullae, haemorrhage and erosions surprising, and they are not infrequently seen in the vulval area. They have also been reported in other sites (Brodey 1967; Glickmann & Silvers, 1972). Response to any form of treatment, including corticosteroids, is usually unsatisfactory but this case showed a marked improvement on ACTH.

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British Journal of Dermatology (1975) **93**, 219.

Reactivation of scabies rash by methotrexate

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Accepted for publication 8 November 1974

SUMMARY

A 13-year-old female was on maintenance therapy for acute lymphoblastic leukaemia. On three occasions she received methotrexate orally and each time this was associated with reactivation of scabies rash. The mechanisms for this phenomenon are discussed.

The occurrence of Norwegian scabies in a patient on an immunosuppressive regime following renal transplantation has been described by Paterson, Allen & Beveridge (1973). We have recently encountered scabies occurring in a patient with acute leukaemia in remission undergoing maintenance chemotherapy but in this instance the clinical picture was different from that seen by Paterson *et al.*

CASE HISTORY

P.McC. (d.o.b. 8.8.60) presented on 17 March 1973 with a 3–4 week history of feeling unwell. On examination the only feature of note was that she was moderately anaemic; a finding confirmed by examination of her peripheral blood which showed the haemoglobin to be 9·6 g/100 ml. The total leukocyte count was 1,300/mm^3, with 40% blast cells and bone marrow aspirate confirmed the diagnosis of acute lymphoblastic leukaemia. She was entered into the Medical Research Council trial for acute lymphatic leukaemia (UKALL II protocol) and following therapy with prednisolone, vincristine and asparaginase, remission was quickly established. Bone marrow taken on 6 April showed a normal pattern. During May she was given 2300 rads to the cranium and 900 rads to the spine as prophylaxis against meningeal leukaemia; also during this time she had five intrathecal injections of methotrexate 10 mg on each occasion.

In early August she was very well and on 10 August she started to take 20 mg methotrexate orally daily for 4 days and was seen again on 22 August. At this time she complained of a painful mouth and rash, both complaints having started at the same time, about 3 days after completion of the course of methotrexate. Her mouth showed many small ulcers on the inner surface of the gums whilst on the trunk and arms there was a fine papular rash. Without specific therapy her condition settled within 7 days, at which time her mouth was quite healed and the only stigmata of the skin lesions were many

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small pigmented spots. On 7 September she started 6-mercaptopurine 100 mg daily and on 14 September she had cytosine arabinoside 80 mg intramuscularly. She had no skin problems at this time and on 28 September again had 20 mg methotrexate orally for 4 days. When seen on 5 October she reported that for 2 or 3 days after taking these tablets the rash was present and was very itchy but no mouth ulcers had occurred and the rash was now fading and was no longer troublesome to her.

She did not have methotrexate again for some weeks during which time she was on prednisolone 55 mg daily for 3 weeks and had two injections of vincristine 1·3 mg intravenously. Throughout this period she had no skin trouble or mouth ulcers.

On 27 October she again started to take methotrexate for 5 days and within 24 h of completing this course she again had very severe mouth ulceration and the rash was once more active. Spontaneous resolution of the rash started within 5 days of completion of therapy and at this point the lesions on her hands were recognized and the skin lesions diagnosed as scabies. The rash at this stage was seen by a dermatologist and consisted of a fine erythematous papular macular rash on the trunk with scattered excoriated lesions on the arms. There were typical burrows on the fingers and an acarus was removed from one of these. Treatment with benzyl benzoate produced rapid resolution. Further exposure to methotrexate in a similar dose on two occasions has not been associated with any further skin lesions.

During all this time she remained very well and except for short periods when the mouth ulcers were present she was at school and enjoying all the usual leisure activities; there has to date been no haematological or clinical evidence of relapse of the leukaemic process.

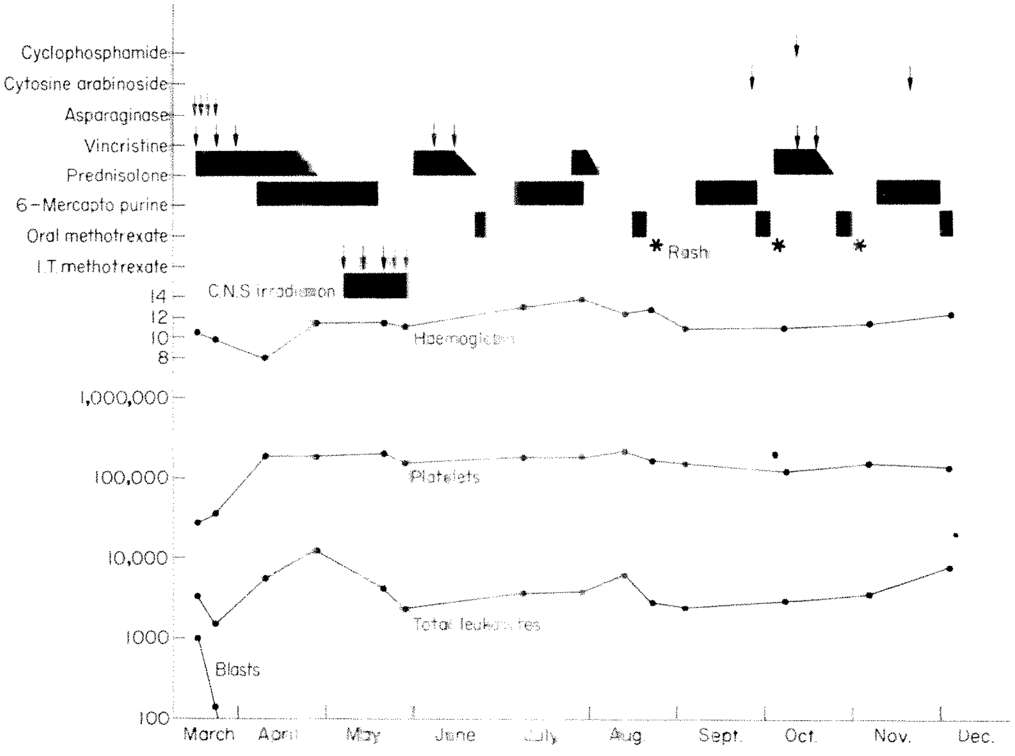

FIGURE 1. Haematological data and treatment regime in relation to appearance of the scabies rash.

DISCUSSION

This patient had her rash on three occasions over a 10-week period and each time it started either whilst on or within 3 days of completing a short oral course of methotrexate, and resolved spontaneously following withdrawal of the drug. In intervals when she was not taking methotrexate she had a variety of other cytotoxic and immunosuppressive agents but no rash occurred. Throughout this time she was unaware of the lesions on her hands which were present in numbers usually seen in patients with scabies. The regime of therapy our patient had was clearly immunosuppressive (Campbell *et al.*, 1973) and one might have expected an acarus infestation to be associated with multiple lesions, as seen by Paterson *et al.* (1973). However in this instance there was no increase in acari but whilst on methotrexate the irritation and papular erythema distant from the infective lesions became evident. It could be that methotrexate either affected the larvae or in some way altered the reactivity of the skin remote from the lesions. One might speculate that the drug may damage larvae and allow release of acarus protein to aggravate the hypersensitivity reaction; we know of no evidence to support this view. It is however known that methotrexate and indeed other cytotoxic agents may affect the reactivity of the skin to physical stimuli to inflammation.

If cyclophosphamide is given before DNCB sensitisation, then the inflammatory response is enhanced (Maguire & Ettore, 1967) and reactivation of erythema following radiotherapy may be associated with taking actinomycin D (Farber, 1958) and hydroxyurea (Sears, 1964). Vogler, Huguley & Kerr (1965) have described patients on methotrexate who showed an exaggerated response to sunlight and the influence of methotrexate on the response to u.v. light has been studied by Moller (1969) in patients receiving this drug for the treatment of psoriasis. He produced acute dermal inflammation by exposure to u.v. light at differing intervals prior to intravenous injection of methotrexate. He found that if the methotrexate was given either 4 days after the u.v. exposure or simultaneously the intensity of the reaction was unaltered. If however the interval between u.v. response and methotrexate was 2 days then the course of the inflammatory response was influenced towards a diphasic curve. This work was a very clear demonstration that methotrexate could reactivate a receding inflammatory response and we would submit that in our patient a similar mechanism was at work, except that in our patient the stimulus to skin reaction was a clinical hypersensitivity rather than a physical one.

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British Journal of Dermatology (1975) 93, 223.

Brief Communications

Fourier analysis of thermal sweating

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Accepted for publication 28 February 1975

A number of reports exist in the literature describing cyclic aspects of human eccrine thermal sweating (Rothman, 1954). Dependent on the technique employed, various frequencies have been observed. These range from monthly cycles associated with menstruation (Taylor *et al.*, 1969) through diurnal cycles (Spruit, 1971) down to cycles of approximately 10 H_z. Cycles of about 1 H_z have been observed using water vapour analysers (Kuypers & Cotton, 1972) and these frequencies are similar to those reported for GSR (galvanic skin reflex or response) cycles.

The 1 H_z cycles are the most obvious in thermal sweating recorded with water vapour analyser systems such as the Meeco and are probably the expression of the activation of the autonomic nerve supply of the gland. The higher frequencies may be due to myoepithelial cell contractions (Nicolaides & Sivadjian, 1972). Since these various frequencies might be expected to represent distinct processes within the total response of thermal sweating, it follows that disorders of sweating might be due to disturbances in any one or combination of these. Consequently it would be very informative to be able to study these frequencies independently. The standard method for selecting individual frequencies from a band of superimposed frequencies is Fourier analysis. The method is adequately described in a number of standard texts and various computer programs have been constructed. We have, therefore, performed manual Fourier analysis on 28 min of continuous sweat record (Fig. 1) and have also done this on-line using a Tektronix digital processing oscilloscope (DPO). The Fourier analysis calculations are extremely tedious to do by hand but are simple with on-line computation. The technique permits the identification of individual frequencies as well as their quantification.

We are now applying the technique to normal subjects under various environmental conditions to establish normal ranges and will follow this with studies on various disorders, such as atopy, in which there is reason to suspect a central or peripheral disturbance in the sweating process.

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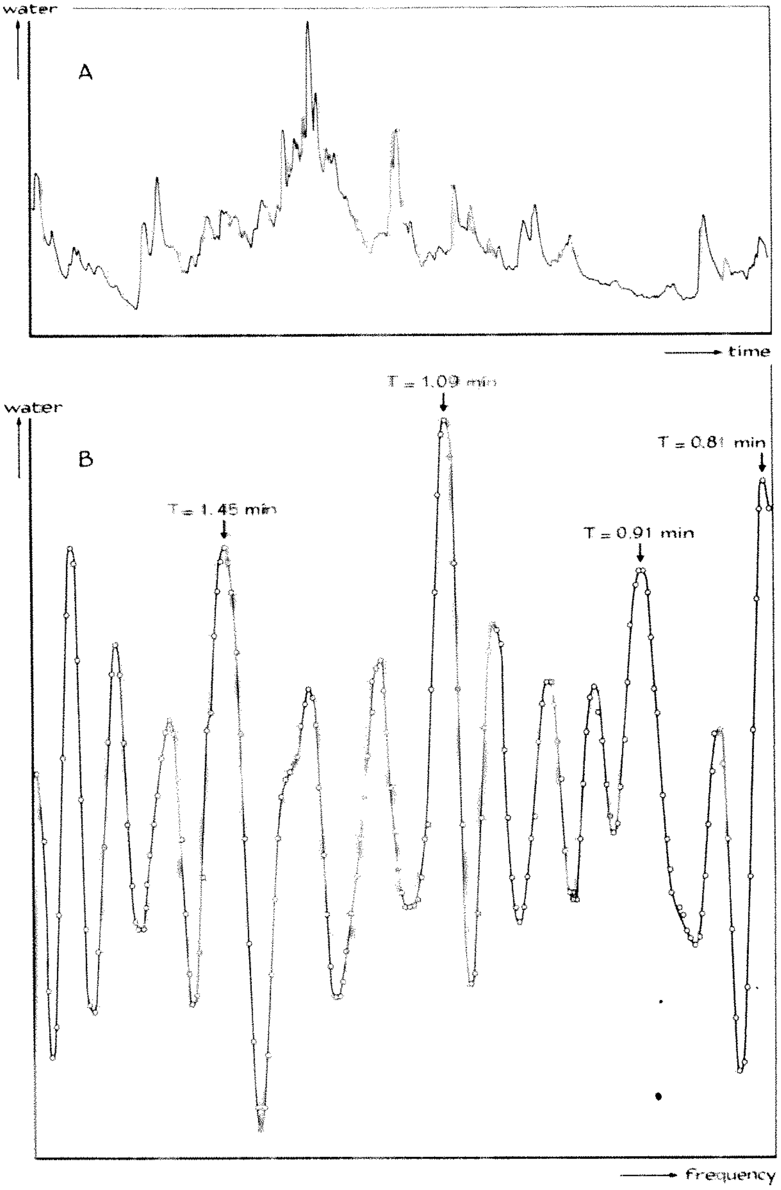

FIGURE 1. (A). Thermal sweating on normal skin of the volar aspect of the forearm measured with the Meeco water vapour analyser at a room temperature of ±30°C in summer.
(B). Fourier cosin transform of 28 min of thermal sweating (A). Sampling frequency, 10/min. T = periodicity in min.

British Journal of Dermatology (1975) **93**, 225.

Impulse cytophotometry in psoriasis

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Accepted for publication 28 February 1975

There seems to be no general agreement in the literature regarding cell cycle parameters in psoriasis; the mitotic duration is reported to be lengthened (Fisher & Wells, 1968) or shortened (Weinstein & Frost, 1968), the DNA synthesis time doubled (Pullmann, Lennartz & Steigleder, 1974; Born & Kalkoff, 1969), or halved (Weinstein & Frost, 1968), the G_1 time shortened by a factor of 10 (Weinstein, 1972) or by a factor of 2 (Pullmann *et al.*, 1974).

Some of these disagreements might be due to variations in the maturity of the psoriatic lesions and inter-individual variations, while others are certainly caused by pitfalls in the methods being used. Another problem is that until recently cell kinetic studies have been very time consuming.

We describe here the application of a rapid method for the analysis of cell kinetic parameters (up to 1000 cells/s) and some preliminary results obtained with it in the kinetics of psoriasis. The instrument used is a pulse cytophotometer (PCP) (Göhde & Dittrich, 1971; Lancet editorial, 1975). Cytophotometers measure light intensities as a function of cell characteristics. In pulse cytophotometry single cells are transported through the field of a microscope-photometer.

In our study we made an epidermal cell suspension from skin biopsies (4mm diameter punch) by treating them with 1% trypsin solution for 30 min at 37°C. The cells were fixed in absolute ethanol and stained with ethidium bromide, a fluorescent dye which stains DNA quantitatively. Each cell produces a fluorescent signal, the intensity of which is proportional to the amount of DNA per cell. The signals are electronically processed and classified into 128 intensity catagories to produce a DNA histogram.

Fig. 1(A) and (B) gives examples of such a DNA histogram from normal and psoriatic skin respectively. In Fig. 1(A) 64,355 and in Fig. (B) 83,699 cells were measured. The area under the major peak (channel number 30) represents cells which contain the 2n amount of DNA (cells in G_1); cells in G_2 and mitosis contain 4n DNA and consequently one can find them around the double channel number. The region in between contains cells in various stages of DNA synthesis (S phase). With a simple arithmetical calculation one can find the percentages of the cells in each phase of the cell cycle.

Table 1 gives the percentages of the cells in the various phases of the cycle and therefore the relative lengths of the phases, if we assume that there is an asynchronous distribution of the germinative population over the cell cycle. These values have to be corrected since not all the epidermal population is proliferative. The cells of the stratum spinosum, for instance, also contain the 2n amount of DNA. One should therefore know the ratio proliferative/nonproliferative cells, or one could attempt to fractionate the cells into two populations. The former problem can be tackled by measuring two parameters with the PCP (e.g. DNA and RNA or DNA and protein) since one might expect different ratios of DNA/RNA or DNA/protein in the differentiated skin layers compared to the basal layer. The absolute time length of the different phases could be determined by synchronization procedures *in vivo* or *in vitro*. Even without these modifications the method already seems to be very promising

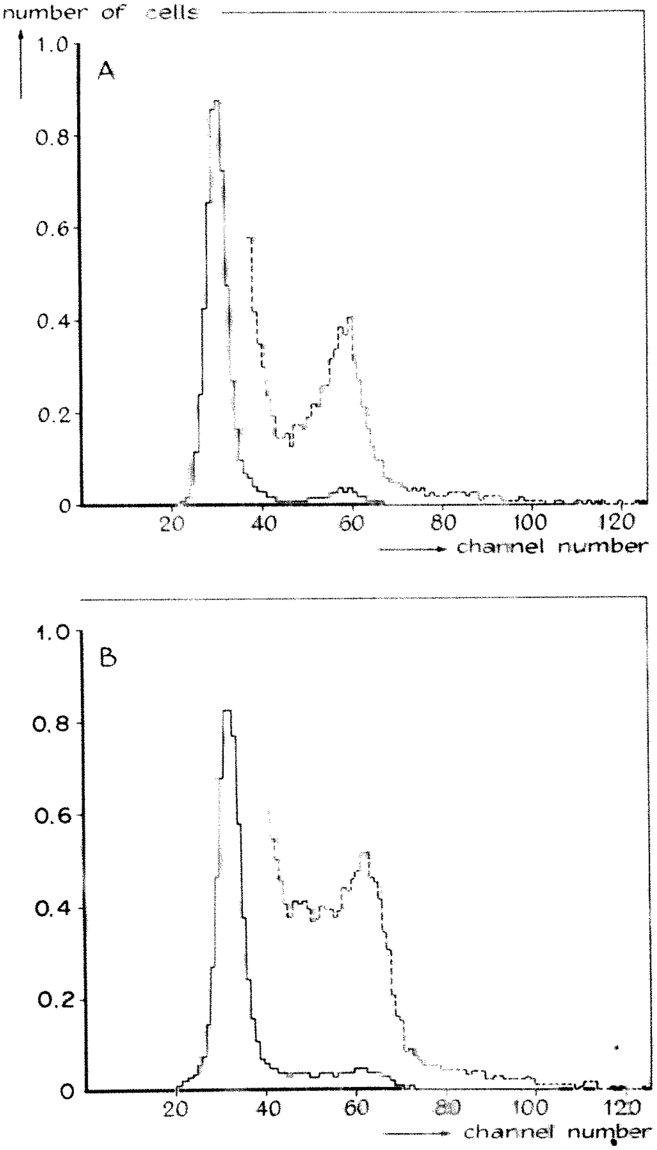

FIGURE 1. DNA Histograms of normal epidermis (A) and of psoriatic epidermis (B). The broken line represents a tenfold amplification of the solid line in each case.

for following the therapeutic effects of antipsoriatic agents. The inconvenience for the patient is minimized since one could work with very superficial 2mm biopsies and the results are obtained very rapidly (within 3 h after the biopsy).

Concerning the cell kinetics, our results seem to indicate a rather short germinative cell cycle both in normal and in psoriatic skin (about 50 h) and probably a lengthened duration of the S phase in psoriasis.

A more extensive report will be published soon.

TABLE 1. % of cells in the different phases of the cell cycle in normal and psoriatic epidermis

	G_1(%)	S (%)	G_2 M(%)
Normal	88	6	6
Psoriatic	79	15	6

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British Journal of Dermatology (1975) **93**, 229.

Vitiligo and jaundice

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Accepted for publication 19 February 1975

Danzig (1953) described the absence of jaundice in the vitiligo patches of a woman with cirrhosis of the liver, and though several possible mechanisms were discussed, no satisfactory explanation was offered. A similar phenomenon was noticed by Pegum (1953) in a patient with vitiligo, treated with mepacrine. Here also the vitiligo patches did not seem yellow, but the fluorescing drug could be demonstrated in the vitiliginous skin, and the vitiligo patches did appear yellow when seen against a white background. Fitzpatrick & Mihm (1971) commented on both cases and wondered if indeed bilirubin is absent in the depigmented skin of patients with vitiligo and jaundice. We would like to describe such a patient in whom the vitiliginous skin was clearly jaundiced.

A 74-year-old housewife was admitted to hospital with severe jaundice, present for about 5 weeks, due to an obstructing pancreatic tumour. The total plasma bilirubin content was 230 μmol/l (normally less than 9 μmol/l). Vitiligo had been present for 34 years, and the patient's mother, brother and a son also had vitiligo.

Localized to the face and on the trunk (around an appendicectomy scar), in the axillae, on the perineum, but mainly on the distal parts of the extremities the patient showed several nummular, partly confluent, patches of clear light yellow colour, contrasting vividly to the other parts of the body, which were dark yellow brown. The sclerae were yellow. The vitiligo patches on the back of the hands and the knees were less yellow than those on the rest of the body.

In the following days the yellow colour increased, in parallel with the plasma bilirubin levels (up to 600 μmol/l). After about 2 weeks an anastomosis between the bile duct and the duodenum was made, resulting in lowering of bilirubin levels and diminution of the jaundice. Just before discharge from hospital, when the bilirubin level had fallen to 100 μmol/l, the yellow colour of the vitiligo patches, especially on the hands, had almost gone. However when the hands were compared with a sheet of white paper the yellow colour was still distinct. The yellow-brown discolouration of the rest of the body remained and the sclerae were still yellow.

In this case we clearly saw that jaundice can exist in vitiligo patches. The difference between our observations and those of Danzig may have been caused by the fact that our patient had much higher levels of plasma bilirubin. Danzig mentioned only one bilirubin value, 6·2 mg/100 ml (= 106 μmol/l), which was approximately the concentration noted in our patient, when her vitiligo patches were almost white.

Jaundice is normally seen when the serum bilirubin level exceeds 2·5–3·0 mg/100 ml (about 60 μmol/l) and so one might have expected to find the vitiligo patches of Danzig's patient and our patient at the time of discharge to be clearly yellow. The relative absence of jaundice could be due to impaired diffusion or fixation of bile pigments in vitiliginous skin, but we feel it is more likely that, as in the case of vitiligo and mepacrine, the phenomenon is an optical effect due to the contrast between the depigmented skin of the vitiligo patches and the surrounding hyperpigmented skin.

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Comment

THE CHOICE AND USE OF EQUIPMENT FOR PHOTOMICROGRAPHS

Sharp black and white photomicrographs of good contrast and gradation can considerably enhance the value of a publication, and can often make a point more clearly than many lines of text. The production of such micrographs is not difficult if suitably matched microscope eyepieces, objectives and condenser and illumination system are chosen and used at their optimum settings. It should be emphasized that a good photomicrograph starts with an atraumatic biopsy of a suitable lesion, proper fixation, dehydration, embedding, and the eventual production of a well-stained section of around 5 μm in thickness. It is advisable to use the same thickness of good quality glass slides for sections intended for photography as thick, poor quality slides may distort the final image. Broadly speaking, most microscope objectives are computed for use with a cover glass of 0·17 mm, and the extra cost of using cover glasses of this guaranteed thickness is more than justified.

While there is no doubt that photomicrographs can be produced by a compound microscope with a camera and lens applied to the eyepiece, these are rarely of sufficient quality for reproduction.

The use of a camera body without lens produces better results and many manufacturers of cameras and microscopes produce adaptors for this purpose. One of the main drawbacks of this type of system is the difficulty in focusing and maintaining sufficient rigidity in the system to obviate camera shake. The majority of good microscope manufacturers supply a special tube which fits on to a monocular body and contains an angled observation tube and eyepiece through which the specimen may be focused, the point of focus in the observation tube corresponding with the focal plane of the film. If such a system is used it is advisable to have the entire microscope–camera combination clamped to a rigid metal rod screwed into a firm base.

It is more common now to purchase one of the photomicrographic systems and these are offered by leading British, German, Swiss and Japanese manufacturers. Perusal of their most attractive coloured brochures indicates that purchase of the system is a simple matter, and it is only when the purchaser is confronted with the long lists of various objectives, eyepieces and condensers available that he realizes that it is far from simple.

The first thing which has to be decided is the negative size. This varies from the standard 35 mm (1 × 1½ in) roll film, 2¼ × 2¼ in roll film, 3¼ × 4¼ in sheet film to 5 × 4 in sheet film. There is no doubt that the larger the negative the less the degree of enlargement required for the final print, and this gives the highest quality. However, for convenience 35 mm film is the most commonly used. With modern fine grain emulsions and developers very high quality results can be obtained with this film size.

There is a wide variety of equipment available today for photomicrography, from a relatively simple compound microscope with a 35 mm camera body mounted on top of it, through more versatile semi-automatic systems to the very expensive, sophisticated, fully automatic systems incorporating a research type microscope capable of phase and fluorescence microscopy. While the type of equipment purchased depends primarily on the kind of work to be undertaken and on the budget available, it is always advisable to buy the best available from the chosen manufacturer. The following remarks apply to any system, from the simplest to the most complex.

Microscope stand

This should be sturdy and preferably of heavy construction so as to minimize vibration which could be transmitted to the camera. A binocular microscope is less tiring to use than a monocular one.

The focusing controls should be convenient to the hands, smooth in operation and completely free from backlash. A low voltage illumination system with a transformer is to be preferred, provided that the illuminator has a lamp condenser and an iris diaphragm (*vide infra*). A built-in illumination system is preferable to an illuminator on a separate stand. An eucentric revolving circular stage is a great asset in aligning the specimen with the optic axis and is to be preferred to the more common square mechanical stage. A substage condenser mount incorporating centering screws is essential. If money is available it is a good investment to buy one of the machines with the camera integral with the microscope body, e.g. the Zeiss photomicroscope.

Condenser

The simple two lens Abbe type condenser is not really suitable for critical photomicrography because of its relatively uncorrected spherical and chromatic aberrations. As the condenser plays such an important part in the image formation, the least which should be purchased is a three lens aplanatic condenser or, if possible, the more expensive achromatic-aplanatic type. It is also important to choose a condenser with a numerical aperture capable of matching the numerical apertures of the objectives chosen. An achromatic–aplanatic condenser with a numerical aperture of 1·0–1·4 will meet the most exacting objective requirements. High aperture condensers of this type usually have a swing-out or screw-out top lens which reduces the numerical aperture to 0·3–0·4. This is necessary to illuminate the field fully when using the lower powered objectives.

Eyepieces

In the past it was considered necessary to have different types of eyepieces for different types of objective construction. It is now customary to use only compensating eyepieces and these are recognized by being marked 'Comp' or merely by the letter K. If these are being used in a stand with an integral camera or in an inclined observation tube (it is necessary to have a monocular tube for this), one eyepiece is capable of individual focusing on to a double hair-line-cross or a double hair-line circle. It is necessary to focus this accurately for each individual user in order to obtain sharp focus at the film plane. The most common magnifications for eyepieces are 6 ×, 8 × and 10 ×, the choice being an individual one. The so-called 'high point' eyepieces are recommended for spectacle wearers, but I personally have not found them either necessary or desirable.

Objectives

There are three main types of microscope objectives for use in transmitted light. Of these the achromatic are the simplest construction and therefore the cheapest. They are free from chromatic aberration in yellow-green light and while perfectly adequate for many routine purposes are not ideally suited for photomicrography, especially when colour film is being used. Next in complexity and cost are the semi-apochromats, or fluorites ('Neofluars'), as they are now called. They have much better colour correction and thus have considerably improved performance and in general are to be preferred to achromats. Their performance, however, is not as good as the fully colour-corrected apochromatic objectives used with compensating eyepieces. While these are expensive, their use together with an achromatic–aplanatic condenser is recommended for critical photomicrography. In recent years there has been a major advance in microscopic objectives with the introduction of flat field objectives which can be recognized by the prefix 'Plan'. Whether achromatic or apochromatic objectives are selected they should always be Planachromats or Planapochromats. If the budget is limited and the majority of the photomicrography is to be in black and white, Planachromatic objectives used in yellow-green light can give excellent results. In theory, if objectives are being used for fluorescence microscopy they should be free of fluorite, but in practice this does not appear to have any real disadvantages. The other aspect of objectives which has a considerable bearing on performance is the numerical aperture. This is a measure of the ability of a lens to resolve fine detail. While a high numerical aperture gives an increase in resolution it also gives a reduced depth of focus and an increase in the curvature of the field of view. The former causes focusing problems unless the section is very thin; the latter causes the edges of the field to be out of focus when the centre is in focus and vice versa. This latter objection has been largely overcome with the provision of Planapochromatic objectives with high numerical apertures, but these are expensive. In general, low power and medium power objectives (4–10 ×) have numerical apertures from 0·1–0·30, while higher power objectives (25–40 ×) have numerical apertures from 0·5–0·95. Apochromatic objectives have higher numerical apertures than fluorite or achromats of the same magnification. The theoretical limit of numerical aperture for a dry lens (used in air) is 1·00, although in practice the upper limit is 0·95. High quality high magnification lenses have a numerical aperture up to 1·4. With these it is necessary to introduce immersion oil, which has a high refractive index, between the cover slip and the front element of the lens (oil immersion lenses) and, in fact, for the best results, between the top lens of the condenser and the undersurface of the glass slide.

It cannot be too strongly emphasized that in order to use objectives of high numerical aperture a condenser of equally high numerical aperture must be used.

Photomicrography

The two great enemies of good photomicrography are (1) dust and (2) finger marks on any of the optical surfaces. It is therefore essential to get rid of any dust around the apparatus and ensure that all the optical components are free from finger marks before commencing to photograph.

Next comes the setting up of the illumination system: this and accurate focusing of the specimen are the most critical aspects of photomicrography. The first step is to line up the lamp in the optical axis of the microscope system. The type of lighting used is known as Köhler's illumination and consists of the accurate control of the light beam before it reaches the specimen. This is why it is so necessary to have a light source with a collecting lens and iris diaphragm, whether as a separate unit or integral with the microscope body. In practice, to satisfy Köhler's principles the objective to be used is first focused on the specimen to be photographed. The lamp iris diaphragm (or field diaphragm) is closed down until its image appears superimposed on the specimen. This image is brought into sharp focus on the plane of the specimen by slightly raising or lowering the condenser. The image of the partially closed diaphragm is then centred in the field of view by means of the centering screws in the substage condenser mount. When this has been achieved the diaphragm is slowly opened until it is just touching the edges of the microscope field of view and any fine corrections to the centering are carried out. The field diaphragm is then opened fractionally so that the image is just removed from the field of view. The next step is to remove one eyepiece and by viewing the back lens of the objective, close the condenser substage iris until the back lens of the objective is four-fifths filled by light. By carrying out these manoeuvres one ensures that the light is centred and that the size of the beam is matched to the numerical aperture of the condenser and objective being used. If the power of the objective is changed, e.g. to a higher or lower power, the lighting must be readjusted as above. The next step is focusing and this is done roughly by the coarse focusing control of the microscope. In the case of a binocular microscope the hair-line cross or circles in one eyepiece is critically focused for the observer and similarly for the single ocular in an inclined observation tube. This is achieved by turning the focusing ring of the eyepiece upwards until it is fully extended, then rotating it downwards onto the cross or circles, the point of focus being when the two lines or circles are clearly separated from each other. It is important to focus these from above in order to relax the accommodation of the eye. If the interpupillary distance on a binocular microscope is moved (altering the tube length) this ocular must be refocused. Critical focusing of the specimen is now undertaken with the fine focusing control of the microscope, bringing the plane of the specimen into the same plane as the hair-line cross or circles.

Exposure

This may be done by trial and error methods using a constant amperage setting for the light source. More accurate results may be obtained by one of the modified exposure meters available or, preferably, by one of the automatic systems available from all microscope manufacturers.

Films for black and white photomicrography

The films most suitable for routine photomicrography are the slow, fine grain, thin emulsion, panchromatic films such as Kodak Panatomic X, Ilford Pan F, and Agfa Isopan F. These may be developed in a general purpose fine grain developer such as D76, although it has been our experience that the use of a high acutance type of developer, such as Paterson's 'Acutol', gives preferable results.

Filters for black and white photomicrography

For haemalum and eosin-stained preparations the use of a narrow band interference green filter, such as Barr & Stroud's MD4 (at 545 nm), greatly enhances contrast and gives a much more 'lively' print. With other stains other coloured filters can be used. If it is remembered that a coloured filter will lighten its own colour and darken its complementary colour it becomes a simple matter to choose the colour of filter required. As coloured filters cut down the amount of light reaching the film, the exposure when using filters has to be increased. The darker the filter, the more exposure required.

Enlargers

Thought should be given to the type of enlarger used in printing enlargements from photomicrographic negatives. Where maximum detail and contrast are desired, a condenser enlarger should be used. This necessitates the development of the film to a lower gamma than if a cold cathode type of diffuse light source is employed. Details of development times to suit the different type of enlarger can be found in the developer manufacturer's literature.

One drawback of the condenser enlarger is that every particle of dust and scratch on the negative is revealed on the final print. The film should thus be handled at all stages with surgical cleanliness and stored in a dust-free atmosphere in scratch-proof envelopes.

Colour photography

Unless some form of electronic flash unit is incorporated into the equipment colour photography must be undertaken with artificial light balanced films, the two most commonly used being Kodachrome A and Agfachrome Professional L reversal colour films. These films are balanced for light of a colour temperature of 3,400°K and 3,100°K respectively, and if exposed in light which is not of this precise colour temperature will show unpleasant colour casts. Most manufacturers will provide information as to the amperage or voltage at which their lamps are to be run to give this colour temperature. The use of colour film balanced for daylight (5,400°K) combined with a correction filter to reduce it to 3,100°K (D to A film) is not recommended other than in an emergency. In general the low voltage tungsten filament lamps are more satisfactory than the modern halogen quartz lamps. Even although the lamp is run at the stated colour temperature, the results may have a slightly reddish or blue cast, and this may be corrected by the use of an appropriate colour correction filter. Exposure of colour film is much more critical than for black and white film and incorrect exposure can also affect the colour balance of the transparency.

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J.A.MILNE

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Book Reviews

Introduction to Chemical Nomenclature. R.S.CAHN (1974) fourth edition. London: Butterworths. pp. 128. Paper. Price £1.80.

From its title, this may seem a curious choice of book to review in these columns. But there can be little doubt that most medical authors will want to own it once they become aware of its existence and recognize its excellence.

Mathematics, physics and chemistry require precise terminology, yet when medical writers touch on these subjects they have a facility for abusing the relevant nomenclature. This can be amusing, more often confusing and sometimes disastrous. This little book will take care of the chemistry.

Dr Cahn has produced a veritable mine of useful information that can be found only with difficulty elsewhere. Not only does his text carry a fully up to date distillate of the universally accepted IUPAC Rules for both inorganic and organic chemical nomenclature, but it also explains the naming procedure and aids the understanding of chemical formulae. Also, this new fully revised and largely rewritten edition offers useful added references.

With this book available, misspelling is no longer excusable (and some currently internationally accepted spellings are indeed surprising) and the spirits need no longer sink when long and daunting chemical names have to be checked and understood. It is to be hoped, too, that even the simple things such as isotopes will be correctly represented and words joined, hyphened or divorced as usage demands.

Any author with pride in his craft will find this book indispensable and will be annoyed that he was unaware of the previous editions. All authors who have to describe chemical terms ought to own, study and use it. Every hospital library should possess it, and retain it in the reference section: potential borrowers should purchase their own.

This book is highly recommended.

J.N.S.MITCHELL

Manual of Contact Dermatitis. SIGFRID FREGERT (1974) Copenhagen: Munksgaard. pp. 107. Price D.kr.48.

This is an excellent manual of practical knowledge of contact dermatitis, written by Dr Fregert on behalf of the International Contact Dermatitis Research Group. It cannot claim to be an academic work as references are few and dogmatic statements abound but any academic who treats patients will find it useful. The book should be read several times over by all trainees in dermatology and by consultants who do not have a major interest in contact dermatitis. It can then be kept and referred to for details of sensitizers. The book will also be enlightening to industrial physicians, to the legal profession and to nurses in patch test clinics.

In 107 pages the mechanism of contact dermatitis, its investigation, details of contact allergens and their source, treatment and prevention are covered. The reader will find the text to be extremely practical; for example the technique of photo-patch testing, including light sources, is fully covered.

It is inevitable and unfortunate that legal issues are frequently involved with contact dermatitis, but in this book it is invaluable to have medico-legal problems discussed including the essentials for a medico-legal report. It might be that in a future edition the four pages of formulae of some chemically defined sensitizers could be omitted in favour of space for references in support of certain statements—for example that hexavalent chromium penetrates the skin more easily than trivalent chromium (p. 20) and to support in detail the controversial view that allergic reactions occur within the mouth (p. 19). In this context it is noted that there is no referral to candidiasis as a common cause of denture sore mouth. There seemed to be only four minor spelling mistakes. This is an excellent little book which sets out clearly and at a practical level the cumulative experiences of the International Contact Dermatitis Research Group.

C.J.STEVENSON

Skin Diseases in the Elderly. JULIAN VERBOV (1974) London: William Heinemann Medical Books. pp. 225, 61 Illustrations. Price £4.40.

The idea and indeed the intention of writing a book on geriatric dermatology is wholly good. More and more our patients are elderly, more and more our society is elderly and more and more our approach to disease and decay is old-fashioned so we should lead the world in the study of the ageing skin! Early attempts at embodying our knowledge may appear a little disjointed as we struggle to discard the attributes of youth in favour of the sagginess of old age . . . not always quite successfully. '*Molluscum contagiosum* is a pox virus infection which is very common in children and young adults but does occur occasionally in the aged.' So, heaven help them, does measles.

This is a little book with some extremely telling and concise descriptions of skin diseases using words to their ultimate effect, and some equally effective black and white illustrations. Trouble comes with the need to translate the ageless approach to that prescribed by the sub-title—'a comprehensive (*sic*) account of clinical geriatric dermatology'—for the sections devoted purely to age changes are rather vague and lifeless. I would rather Dr Verbov had painted more sharply a picture of our ageing selves, and of that which he knew we were capable of dermatologically speaking.

And dermatologically speaking let none of any generation ever, ever commit such a sentence ever ever again: 'In chronic urticaria psychogenic factors such as tension, worry, fatigue and marital disharmony are important but foods, drugs, inhalants and systemic diseases including systemic lupus erythematosus, renal disease, hepatic disease, visceral carcinoma, blood dyscrasias and metabolic disorders should be excluded'. From bumps in the night, Good Lord deliver us.

R.D.SWEET

Disorders of the Autonomic Nervous System. R.H.JOHNSON and J.M.K.SPALDING (1974) Oxford: Blackwell Scientific. pp. ix+300, illustrated. Price £6.50.

Johnson and Spalding have successfully combined a scholarly approach to diseases of the autonomic nervous system with a plain style of writing, accompanied by interesting historical notes, which make very agreeable reading. Five chapters deal with autonomic control of the circulation and its dysfunction—a subject where the authors have themselves made important contributions to the literature. There are also two introductory chapters on anatomy and pharmacology and two on central body temperature regulation. The remainder deals with individual systems.

The section on vascular disorders of the limbs succinctly defines our ignorance of the mechanism of Raynaud's disease and other cold-sensitivity states. It is depressing that, apart from recent work eliminating increased sensitivity of central thermoregulation as an important factor in Raynaud's disease, we know little or nothing more than did Lewis in the 1930's.

More informative, are discussions in the same chapter of the circulatory disturbances in paralysed limbs. The influence of metabolism and heat production in healthy muscle on the dynamics of the circulation in the overlying skin is of especial interest. The axon reflex flare is described in the usual way but the authors, who have themselves done work on this phenomenon, do not discuss the most puzzling aspect of the reflex—that it implies release of transmitter substance by *sensory* nerve terminals innervating vessels.

The chapter on sweating is very good and describes methods of clinical investigation of dyshidrotic states. There is a particularly full account of gustatory hyperhidrosis and its mechanism. One would have liked to have had the authors' views on cholinergic urticaria.

The book is very well produced and illustrated and the index is satisfactory. It deserves to be widely read by dermatologists.

M.W.GREAVES

Organpathologie. Edited by W.DOERR (1974) vol. 3. Stuttgart: Georg Thieme. pp. 390, 363 illustrations. Price £17.50.

This is the third volume of a handsomely produced multi-author work in the Aschoff tradition, treating the whole of pathology by organs or systems. Dermatology is considered in 23 pages, 7 more than the eye is accorded and a dozen less than the ear. The section on the skin was written by Professor Günther Schalloch, Director of the Pathological Institute of the Faculty of Clinical Medicine in Nürnberg. Anyone who has tried to prepare a *précis* of skin pathology for a 'general' reader and has been subject to severe editorial constraints of space and time, will acknowledge that the author has achieved a small miracle of compression and clarity. (The few illustrations he has been permitted are mostly disappointing.) This chapter provides an overview, so to speak, of dermatopathology but for the dermatologist in training or practice more detail and differential diagnosis are necessary.

P.J.HARE

British Journal of Dermatology (1975) **93,** 238.

News and Notices

INVESTIGATIVE GROUP

British Association of Dermatologists

The next meeting of the group will be held at the University of Leeds on Friday and Saturday 23 and 24 January 1976. Those requiring accommodation should write to Dr W. Cunliffe, Department of Dermatology, Leeds General Infirmary. Members wishing to submit papers should send standard abstracts (details are being precirculated) to Professor Greaves, Institute of Dermatology, Lisle Street, London. W.C.2. not later than 13 October 1975.

INSTITUTE OF DERMATOLOGY

University of London

Applications are invited from those wishing to be considered for inclusion in the Diploma of Dermatology Course commencing 6 October 1976. Applications should be submitted before 30 November 1975, and applicants will be notified in March 1976 as to whether or not their application has been successful. Application forms may be obtained from: the Dean, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ.

Post-Graduate Medical School, University of Cambridge

The annual introductory course on 'Biology of the Skin' will be held at Fitzwilliam College from 7 to 12 December 1975. Enquiries and applications should be sent to: The Secretary, Cambridge University Medical School, Hills Road, Cambridge.

Rudolph Ellender Medical Foundation

The Rudolph Ellender Medical Foundation will be presenting its Second Annual Dermatocryosurgical Seminar on 4 and 5 October 1975. Further information may be obtained from the Foundation at: Rudolph Ellender Medical Foundation, Inc., Roof, Delta Towers, New Orleans, Louisiana 70112, U.S.A.

St John's Hospital for Diseases of the Skin

Research Grants totalling £200,000 have been received from the MRC and Wellcome Trust. They will be used to establish and support new skin pharmacology laboratories in the Institute's Homerton, E.9. branch and to enable research to be carried out on pharmacological mechanisms of skin inflammation.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from a countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 93, Number 2, August 1975

CONTENTS

PAGE

Clinical and Laboratory Investigations

Naevus Superficialis Lipomatosus: A Clinicopathological Report of Twenty Cases 121
EDWARD WILSON JONES, RONALD MARKS AND D. PONGSEHIRUN

Reactions to Aspirin and Food Additives in Patients with Chronic Urticaria, Including the Physical Urticarias 135
H. M. G. DOEGLAS

One Hundred Twin Pairs Patch Tested with Primary Irritants 145
ROLF HOLST AND HALVOR MÖLLER

Hypersensitive and Insensitive Extremities: The Dermatological Aspects 151
SAMUEL L. MOSCHELLA

Temperature Dependence of Water Content of Stratum Corneum 159
T. S. SPENCER, C. E. LINAMEN, W. A. AKERS AND H. E. JONES

Intraendothelial Tubular Aggregates in Experimental Wounds: Ultrastructural Study 165
R. A. J. EADY AND G. F. ODLAND

Long-spacing Collagen in Skin Biopsies from Patients with Lepromatous Leprosy 175
ROSA P. EDWARDS

Hazards of Steroid Therapy in Hepatic Failure 183
W. J. CUNLIFFE, J. L. BURTON, G. HOLTI AND V. WRIGHT

Tissue Nickel Levels and Nickel Dermatitis. I. Nickel in Hair 187
S. A. KATZ, H. J. M. BOWEN, J. S. COMAISH AND M. H. SAMITZ

Physiological and Environmental Control of Gram Negative Bacteria on Skin 191
M. E. MCBRIDE, W. C. DUNCAN AND J. M. KNOX

Pruritus Vulvae: Treatment by Multiple Intradermal Alcohol Injections 201
G. D. WARD AND J. R. SUTHERST

Pharmacology and Treatment

Simplified Dithranol Treatment for Psoriasis 205
R. H. SEVILLE

The Absorption of Hexachlorophane from Ultralanum Ointment 209
PATRICK G. T. BYE, WARWICK MORISON AND ELLEN L. RHODES

Case Reports

Generalized Bullous and Haemorrhagic Lichen Sclerosus et Atrophicus: Marked Improvement with ACTH 215
ADRIANO DI SILVERIO AND FERDINANDO SERRI

Reactivation of Scabies Rash by Methotrexate 219
D. BURROWS, J. M. BRIDGES AND T. C. M. MORRIS

Brief Communications

Fourier Analysis of Thermal Sweating 223
D. W. K. COTTON, G. J. DE JONGH AND F. A. J. THIELE

Impulse Cytophotometry in Psóriasis 225
F. W. BAUER AND R. M. DE GROOD

Vitiligo and Jaundice 229
H. NEERING

Comment

The Choice and Use of Equipment for Photomicrographs 231
J. A. MILNE

Book Reviews 235

News and Notices 238

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2. Archs. Derm. **86**, 608, 1962
3. Brit. J. Derm. **90**, 197, 1974

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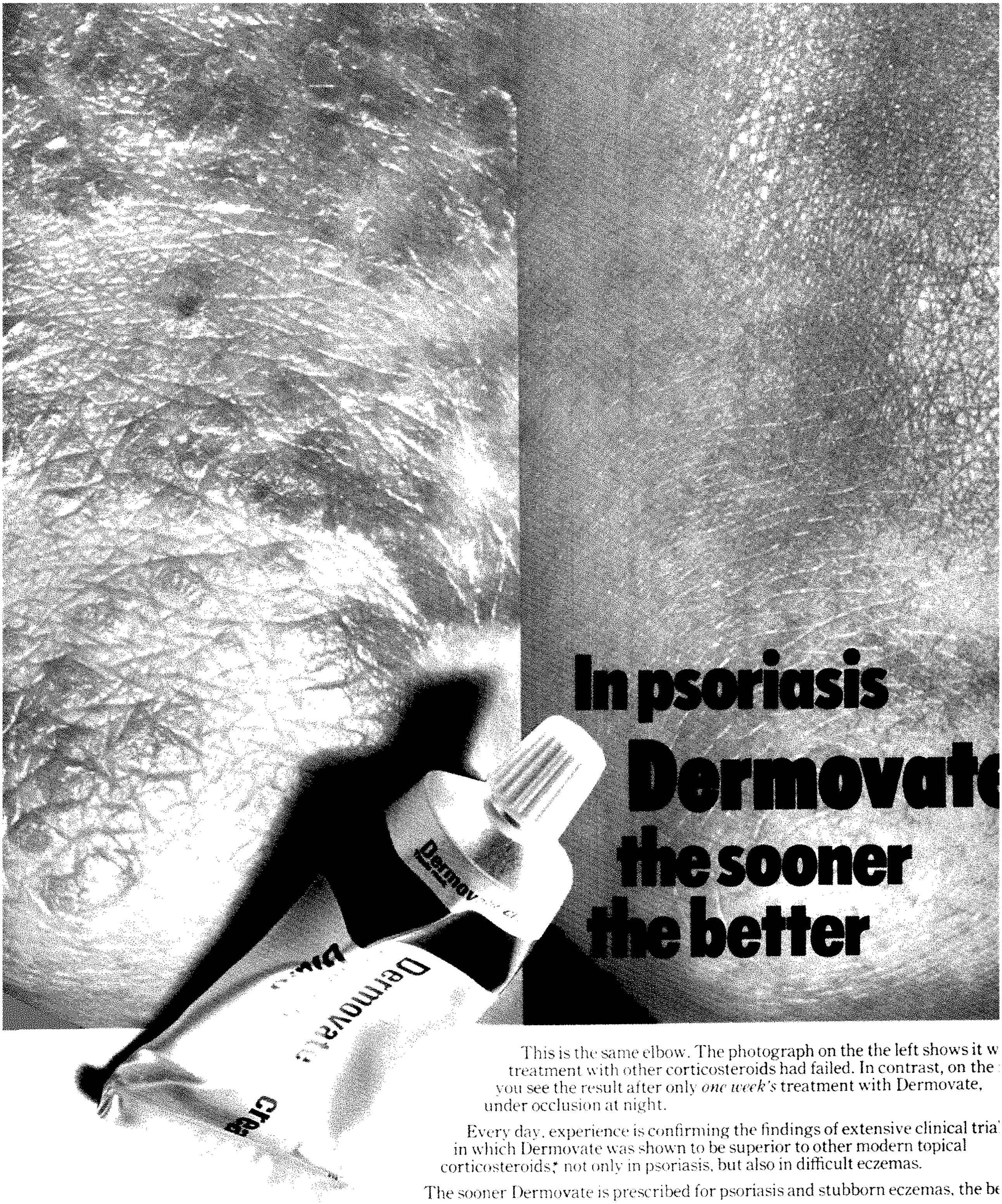

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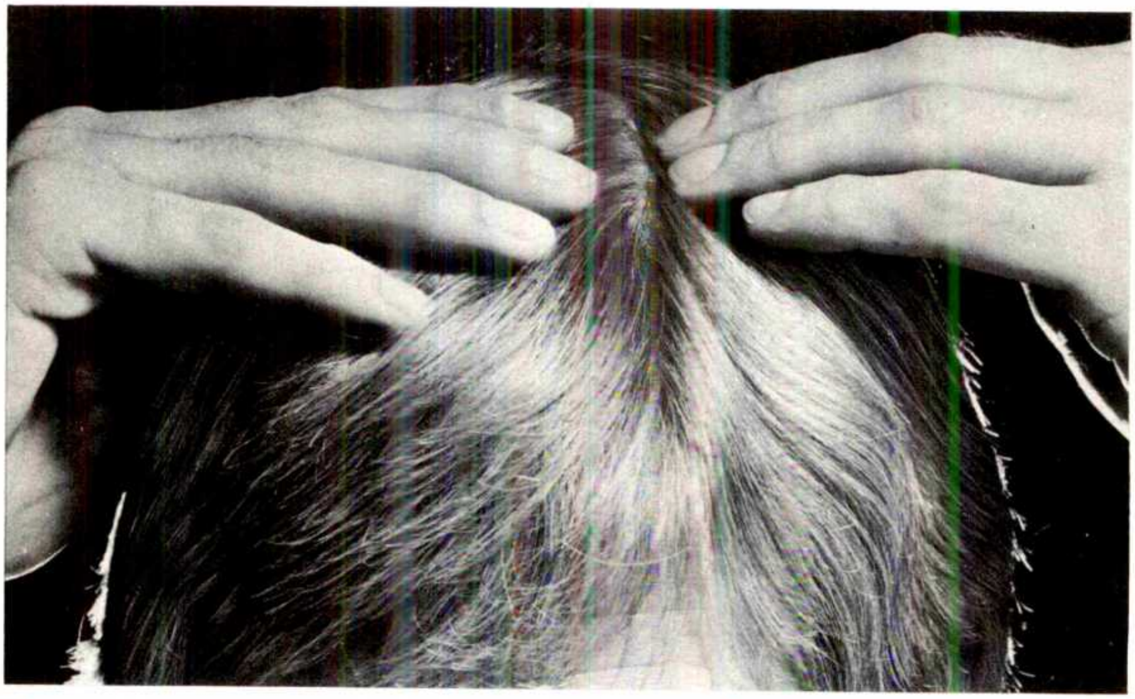

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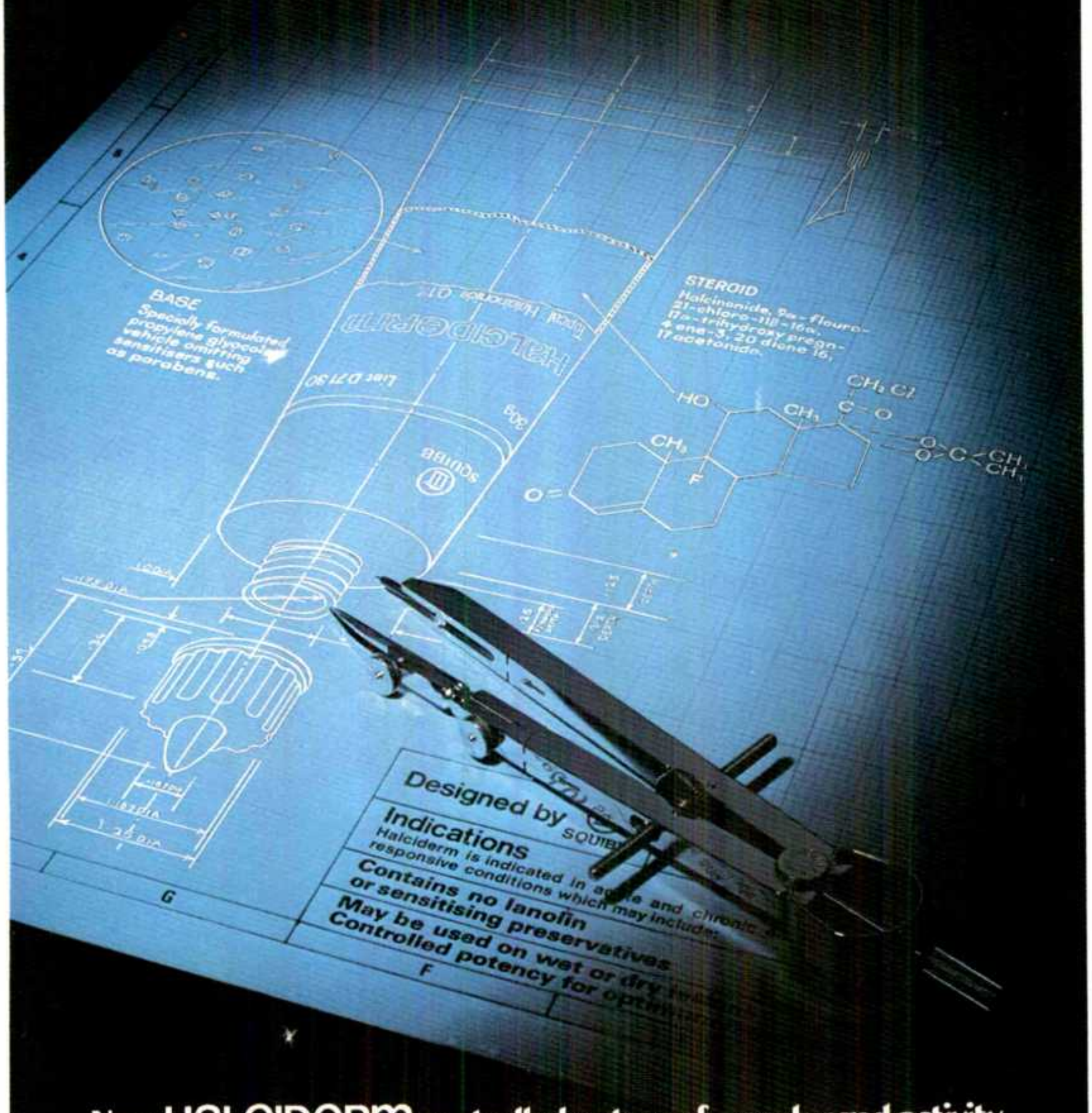
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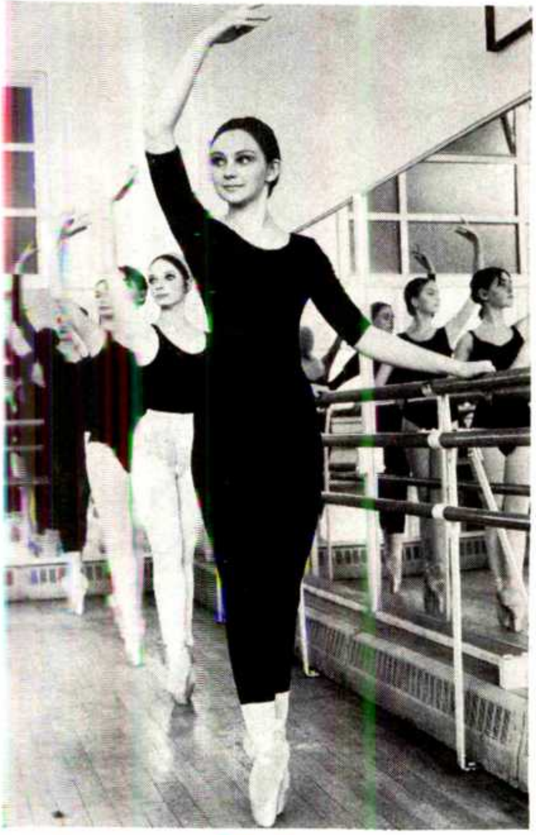

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1. Almeyda, J. and Burt, B.W., *Brit. J. Derm.* (To be published) 2. Harries, E. H. L. and Jacoby, R. H. (To be published)

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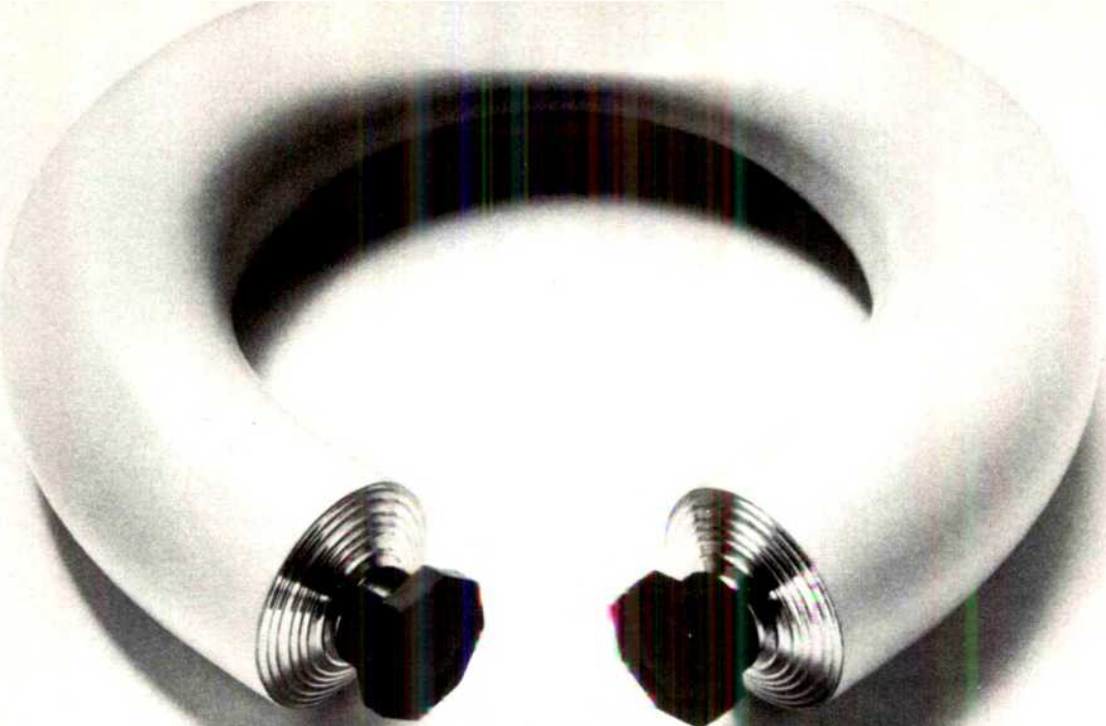

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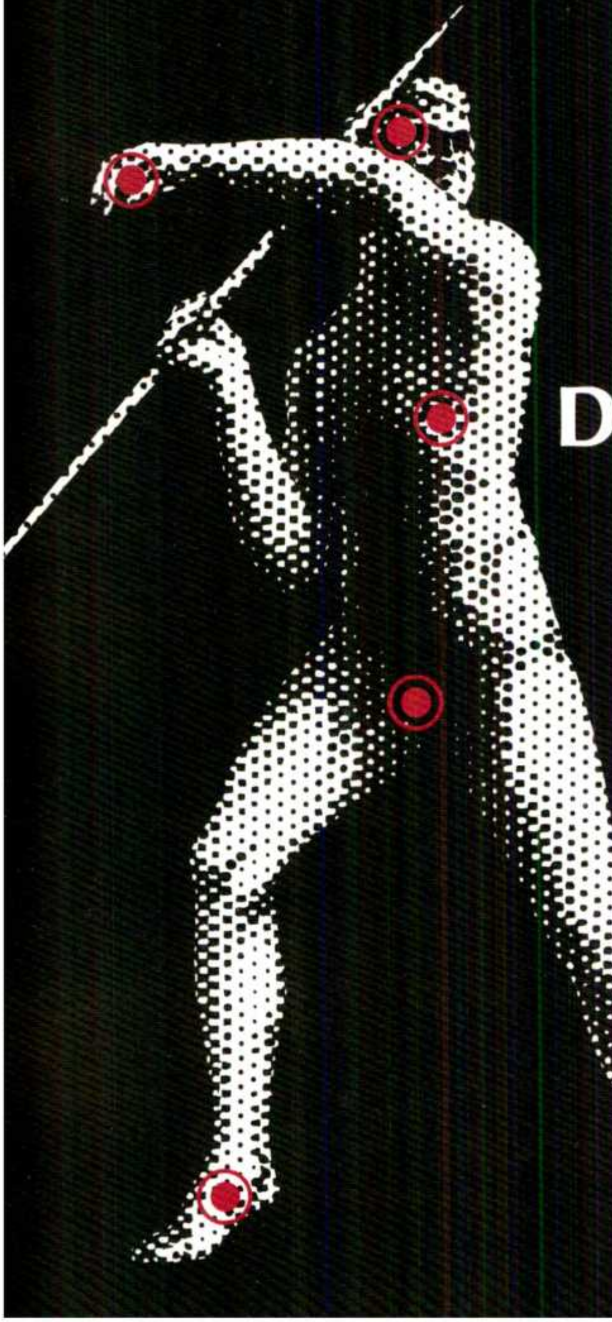

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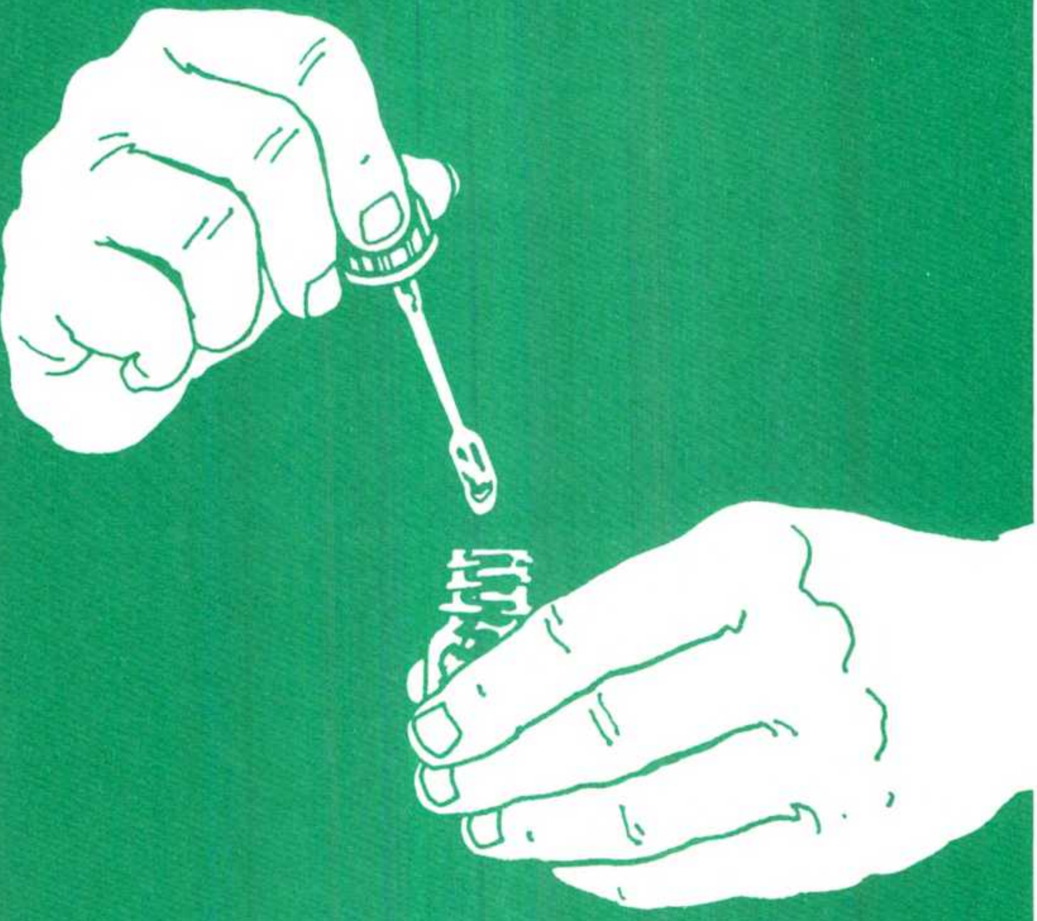

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Index Vol. 150, No. 2, 1975

Toribio, J. and Quiñones, P.A. (Valladolid): Inflammatory Linear Verrucose Epidermal Nevus

Pandhi, R.K.; Bedi, T.R., and Dhawan, I.K. (New Delhi): Leiomyosarcoma of the Labium majus with Extensive Metastases

Cotton, D.W.K.; Hasselt, P. van, and Bergers, A.M.G. (Nijmegen): Nature of the Sweat Glands in the Hairy Skin of the Beagle

Sood, V.D.; Pasricha, J.S., and Kandhari, K.C. (New Delhi): Elimination of Circulating Antibodies. Attempts to Eliminate Antibodies Circulating in Rabbits using a Fraction of Normal Human Epidermis

Jurnovoy, J.B.; Forbes, P.D., and Johnson, W.C. (Philadelphia, Pa.): Tritiated Thymidine Uptake in Pig Skin. Comparison of *in vivo* and *in vitro* Techniques

Brenner, S.; Krakowski, A.; Levtov, O.; Heldenberg, D.; Werbin, B., and Tamir, I. (Tel-Aviv): Serum Lipids in Patients with Psoriasis

Degreef, H.; Verhoeve, L., and Cutsem, J. van (Louvain/Beerse): Miconazole Nitrate in the Treatment of Dermatomycoses

Hejazi, N. (Detroit, Mich.): Umbilical Polyp: a Report of Two Cases

Smeenk, G. (Deventer): Influence of Local Triamcinolone Acetonide on Patch Test Reactions to Nickel Sulfate

García-Pérez, A. and Carapeto, F.J. (Salamanca): Pretibial Epidermolysis bullosa: Report of Two Families and Review of the Literature

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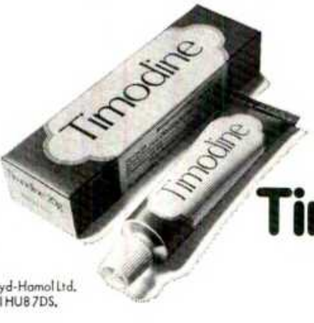

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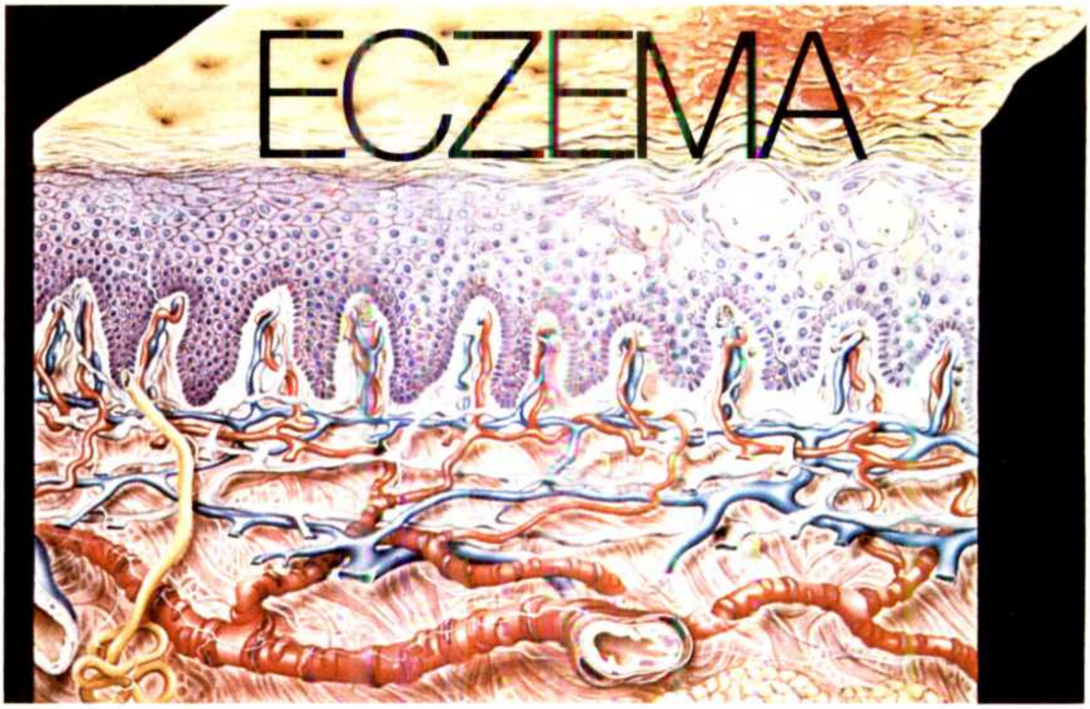

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1) *British Journal of Dermatology*, (1972), **86**, 310.
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British Journal of Dermatology (1975) **93**, 239.

Clinical and Laboratory Investigations

Intra-epidermal carcinoma of the eyelid margin

D.I.McCALLUM*, P.D.C.KINMONT, D.WYNN WILLIAMS, R.E.COTTON
AND M.A.WROUGHTON

Combined Clinic, General Hospital, Nottingham

Accepted for publication 2 December 1974

SUMMARY

Twenty-two cases of intra-epidermal carcinoma of the ciliary margin have been diagnosed in a 15-year survey. The clinical appearance is variable, resembling in some instances a benign warty lesion, and in others a fully developed squamous cell carcinoma. As the lesion grows it produces keratotic plugging of the lash follicles and nodules on the lid margin; in due course this results in loss of the related cilia. When dermal invasion occurs the resultant squamous cell carcinoma is potentially dangerous because of the involvement of the adnexal structures, which on the ciliary margins are particularly large and penetrate deeply into the lid substance. Adequate biopsy which includes at least one lash follicle is essential for accurate diagnosis, and treatment requires complete excision with a reasonable margin for safety. Of the twenty-two patients, nineteen were male and three female; fourteen of the men involved had handled oils and greases for prolonged periods.

Tumours of the eyelid are not uncommon. In a survey of cases seen at the Nottingham-Derby Combined Clinic (1959-1973) the following neoplasms affecting the lids were noted (Table 1):

TABLE 1. The incidence of tumours of the eyelid and of the whole skin

	Upper lid	Lower lid	Total	All areas		Total
				Male	Female	
Basal cell carcinoma	48	270	318	1619	1575	3194
Superficial basal cell carcinoma	—	1	1	22	28	50
Squamous cell carcinoma	2	6	8	480	180	660
Intra-epidermal carcinoma not involving the ciliary margin	2	11	13	219	174	393
Intra-epidermal carcinoma involving the ciliary margin	13	9	22			

---

* Present address: Raigmore Hospital, Inverness, Scotland.

The twenty-two cases of intra-epidermal carcinoma involving the ciliary margins will be discussed in some detail. Treatment of the other cases has not presented any special difficulties. The great majority have been excised, and where necessary the defect grafted by the plastic surgeon; recurrence rate has been negligible, and the cosmetic result very good.

We consider that intra-epidermal carcinoma of the eyelid margin is potentially dangerous, as compared with similar lesions elsewhere on the body. This altered prognosis may be due in part at least to

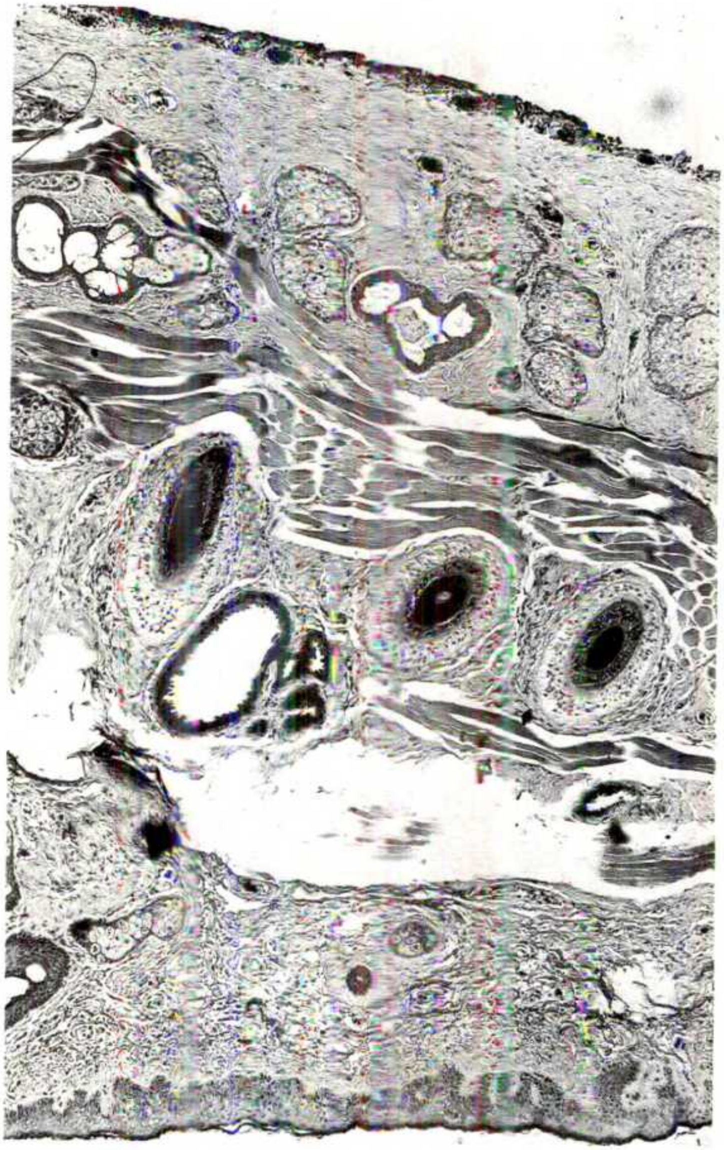

FIGURE 1. Section taken across a normal upper eyelid parallel to the free margin and showing conjunctiva superiorly and skin surface inferiorly. Lash follicles are seen centrally, deeply embedded in the collagenous tissue of the tarsal plate. Sebaceous glands are numerous throughout the section and sweat ducts and glands are also noted in the central zone. (Haematoxylin and Eosin × 8.)

(a) the distinctive anatomy of the eyelid and (b) the behaviour of intra-epidermal carcinoma (Bowen's disease) in relation to the skin appendages.

(a) *Anatomy of the eyelid margin*

The cilia are arranged in two or three irregularly placed rows and are longer and more numerous in the upper lid. The follicles of the cilia extend 1·5–2·5 mm into the fibrous tissue of the lids. Certain cutaneous structures are also modified, the sebaceous glands (Zeis glands) and the sweat glands (glands of Moll) having distinctive features. The sebaceous glands of Zeis are present on the lid margin in association with the cilia; they are larger than average, and open into the ciliary follicle by a short, wide duct. The sweat glands of Moll are of an unusually large type, lying between the cilia and extending into the substance of the lid as deeply as the tarsal plate. Their ducts open either into the ducts of the Zeis glands, or directly into the ciliary follicles, close to the surface (Fig. 1).

(b) *Intra-epidermal carcinoma (Bowen's disease) in relation to the skin appendages*

Pinkus & Mehregan (1969) discussing changes that occur in association with Bowen's disease state: 'The balance between dysplastic epidermis and adnexal epithelium often is disturbed in Bowen's disease to the disadvantage of the adnexa. Instead of forming hyperkeratotic umbrellae the follicular epithelium often just reaches the skin surface. In many cases dyskeratotic epidermis invades ostial territory and may replace follicular epithelium all the way down to the sebaceous duct without breaking the basement membrane.'

The differential diagnosis of tumours occurring on the lid margins includes warts and warty naevi, cellular naevus, cyst, seborrhoeic keratosis, chalazion, basal cell carcinoma, squamous cell carcinoma, Zeis gland and Meibomian gland carcinoma, extra-mammary Paget's disease, and the group of intra-epidermal carcinomata that form the subject of this paper. Differentiation between these lesions may be difficult; of the twenty-two proven cases of intra-epidermal carcinoma listed in Table 2 the initial histological report on eight was benign keratosis, mainly because of inadequate biopsy. Fifteen other lesions, clinically considered to be intra-epidermal carcinomata, were reported histologically to be benign keratoses, i.e. solar or senile keratoses. Of these, two are still considered to be carcinoma *in situ* and are under close surveillance; further and adequate biopsy in such cases will probably be required to establish the diagnosis. Early lesions of intra-epidermal carcinoma of the eyelid may look like warts or simple keratoses, i.e. benign solar or senile keratoses (*vide* Case 1). Careful follow-up of such lesions is indicated to detect evidence of extension and involvement of the lash follicles. If this occurs adequate biopsy and histopathological examination are essential. When the ciliary follicles are plugged with keratin, and more especially when the ciliary margin becomes nodular, biopsy excision will establish the diagnosis and provide effective treatment.

Twenty-two cases of intra-epidermal carcinoma are tabulated (Table 2) in chronological order of referral; five are dealt with in greater detail to demonstrate the stages of development from the relatively innocuous to the lethal.

Case 1 (*Table* 2, *No.* 12)

A male storekeeper, aged 34 years, presented with a keratotic lesion of the left lower lid of a year's duration, and of benign appearance (Fig. 2). His work as a storekeeper brought him in contact with oils. The lesion was raised, warty, 0·7 cm in diameter and impinged on to the conjunctival margin. It was removed under local anaesthesia by a wedge excision.

Histology. 'Surface intra-epidermal malignant change is seen throughout the lesion, extending to the conjunctival margin. One lash follicle only shows very slight involvement near the surface, but deep penetration of adnexal structures is not seen.'

TABLE 2

Date of referral	Patient no.	Sex	Age on referral	Occupation	Oil contact	Duration of lesion prior to referral	Site	Histopathology	Treatment and results
1960	1	M	52	Garage engineer	+	2–3 year	R.u. & l.	I.E.Ca - S.C.C	Repeated surgery, died of metastases.
1961	2	M	73	Plate layer and car park attendant	+	3–4 year	L.u.	I.E.Ca.	Excised—no recurrence
1963	3	F	45	Housewife	o	1 year	R.u.	I.E.Ca.	Excised—no recurrence
1964	4	M	58	Dustman	+	6 months	L.l.	I.E.Ca.	Excised—no recurrence
1965	5	M	64	Shunter	+	10 year	R.u.	I.E.Ca.	Excised and recurred on several occasions
1967	6	M	56	Taxi proprietor	+	6 month	L.u.	I.E.Ca.	Excised—no recurrence
1968	7	M	60	Fitter	+	1 year	L.l.	I.E.Ca.	Excised—no recurrence
1969	8	M	61	Joiner	+	3 year	R.u.	I.E.Ca.	Excised twice—no further recurrence
1969	9	M	61	Self employed	o	6 week	R.l.	I.E.Ca.	Excised twice—no further recurrence
1970	10	M	62	Carriage cleaner (British Rail)	+	2 year	L.u.	I.E.Ca & S.C.C.	Excised—no recurrence
1970	11	M	65	Dock worker	o	months	R.l.	I.E.Ca.	Excised—no recurrence
1971	12	M	34	Store keeper	+	1 year	L.l.	I.E.Ca.	Excised—no recurrence
1971	13	M	59	Lamp room attendant	+	3 year	R.u.	I.E.Ca.	Excised—no recurrence
1972	14	F	51	Housewife	o	1 month	R.u.	I.E.Ca.	Excised—no recurrence
1972	15	M	58	Bus driver	+	1 year	L.u.	I.E.Ca.	Excised—no recurrence
1972	16	F	45	Shorthand typist	o	6 month	R.u. & l.	I.E.Ca & S.C.C	Excised—no recurrence
1972	17	M	64	Labourer	o	6 month	R.l.	I.E.Ca.	Excised—no recurrence
1973	18	M	61	Printer	+	month	R.l.	I.E.Ca.	Excised—no recurrence
1973	19	M	44	Bakery salesman	o	8 month	L.u.	I.E.Ca.	Excised—no recurrence
1973	20	M	78	Ret. railway wkr.	+	10 year	L.u.	I.E.Ca.	Excised—no recurrence
1973	21	M	88	Ret. timekeeper.	+	5 year	L.l.	I.E.Ca.	Excised—no recurrence
1974	22	M	67	Ret. teacher.	o	5 month	L.u.	I.E.Ca.	Excised—no recurrence

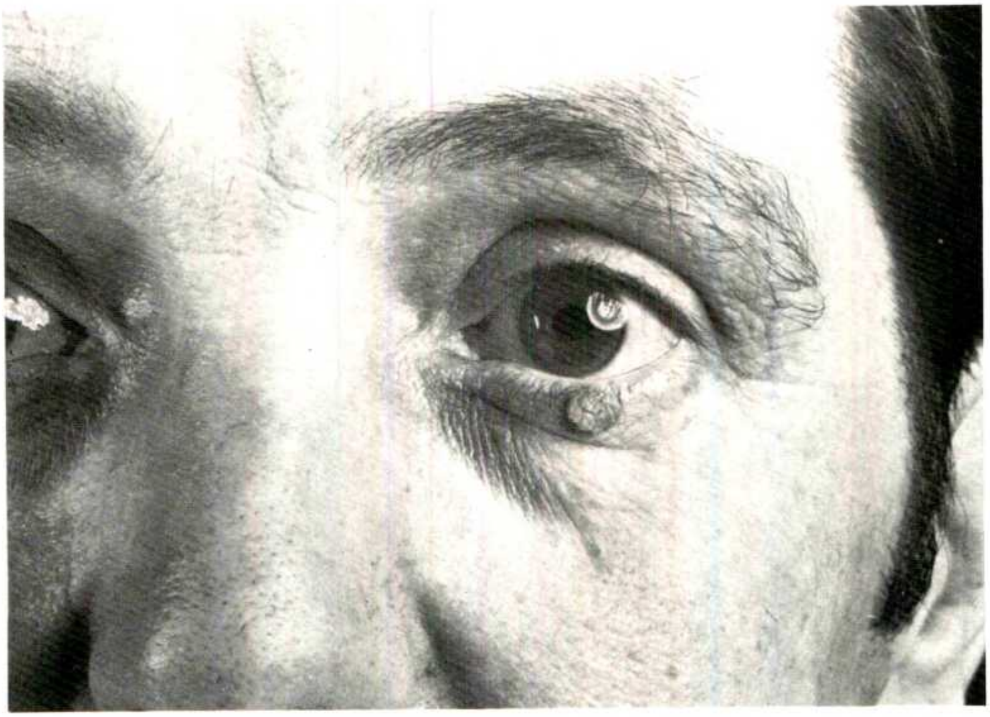

FIGURE 2. Case 1 with warty lesion on left lower lid.

Case 2 (*Table* 2, *No*. 8)
A male joiner, aged 61 years, had on the right upper lid a warty lesion which had been present for 3 years. Curette biopsy showed no evidence of malignancy. Five weeks later he had a keratinizing lesion 2 cm in diameter covering two-thirds of the eyelid. The contour was roughly circular, but the lesion extended along the ciliary margin with a series of warty nodules. The upper lid and the whole ciliary margin were excised.

Histology. 'Intra-epidermal malignant change extends into the hair follicles, some of which show retention cyst formation. Other follicles show a more florid type of intra-epidermal carcinoma which also involves the upper part of the pilosebaceous ducts.'

Further excision was undertaken 3 months later. Histology showed intra-epidermal carcinoma without invasion, and excision appeared complete.

Case 3 (*Table* 2, *No*. 9)
A male shopkeeper, aged 61 years, claimed that the lesion of the right lower lid had been present for only 6 weeks before referral. He had had a basal cell carcinoma on the right side of the nose treated with X-rays 25 years previously. There was a crusted lesion 2·4 × 1·0 cm on the right lower eyelid adjacent to the irradiated area. The eyelashes of the right lower lid were absent. There were numerous keratoses on the scalp and left temple. A clinical diagnosis of intra-epidermal carcinoma involving the lash follicles was made, and this was confirmed by biopsy. The eyelid and lid margin were excised and the lid reconstructed.

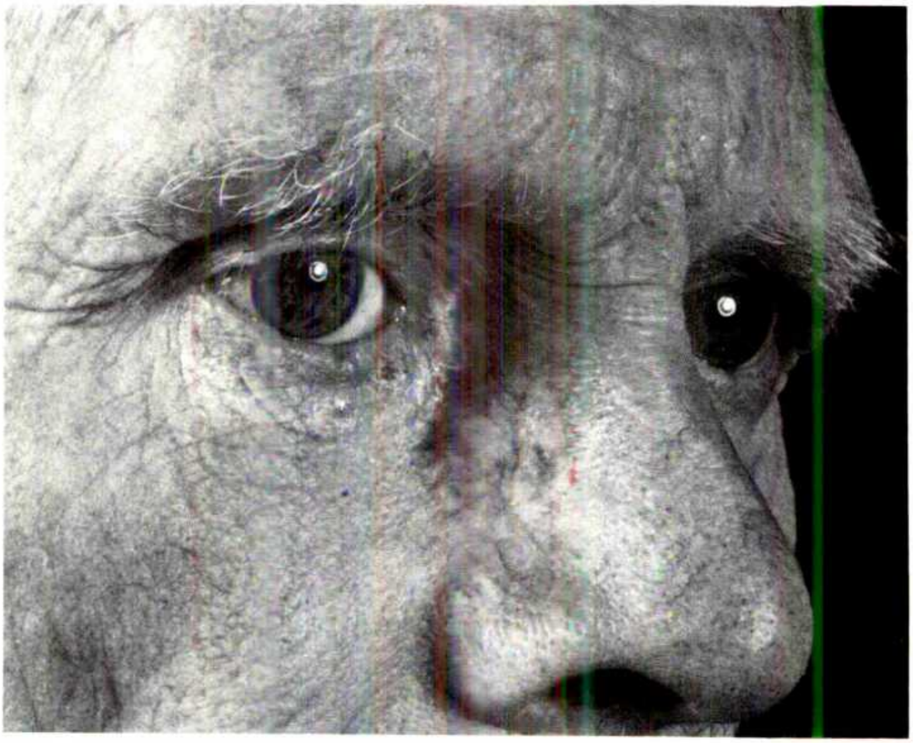

FIGURE 3. Case 3 showing involvement of right lower lid and radiotherapy scarring of right side of nose.

Histology. 'Many sections show florid intra-epidermal carcinoma which is most marked in the lash bearing area and extending deep into the follicles. The conjunctiva is also involved. It is difficult to know if excision is complete as the changes are so widespread.'

Twenty months later a small keratosis was seen just below the lid margin on the grafted area of the right lower lid. This enlarged to 0·4 cm in diameter in 2 months and was excised. Histology showed ulceration in relation to an area of intra-epidermal carcinoma without evidence of invasion. Excision was complete. He has remained well since.

Case 4 (*Table* 2, *No*. 5)

A male shunter, aged 64 years, had a warty lesion on the right upper lid which had been present for 10 years. It was first treated by carbon dioxide snow, but continued to increase in size. In November 1965 the inner half of the right upper lid was covered by warty keratotic tissue which involved the lid margin. The patient complained of constant pain and irritation from conjunctival involvement. Biopsy of this area showed intra-epidermal carcinoma without dermal invasion. The upper eyelid, the ciliary margin and the affected part of the conjunctiva were removed by wedge excision and the lid reconstructed.

Histology. 'A keratotic and papillary squamous cell lesion with some intra-epidermal carcinomatous change; no evidence of invasion; excision appears complete.'

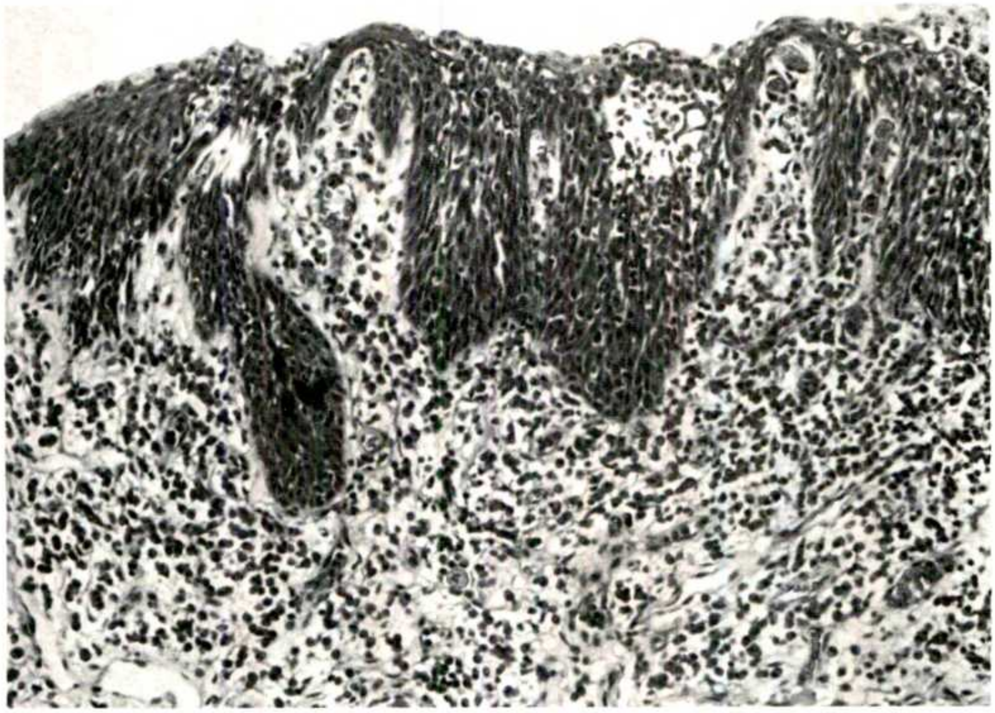

FIGURE 4. Florid intra-epidermal carcinoma with marked pleomorphism and downgrowths (Case 3). (Haematoxylin and Eosin × 30.)

Three months later the whole upper lid became nodular, red and crusted. This was considered to be invasive squamous carcinoma and the whole upper eyelid was excised and replaced. He is still under supervision and remains well.

Histology. 'Extensive intra-epidermal carcinoma of the ciliary margin of the upper lid extending on to the conjunctiva. No evidence of invasive growth.'

Case 5 (*Table* 2, *No*. 1)

A male motor engineer, aged 52 years, was first seen in August 1960 with a keratotic lesion of 2–3 years' duration on the lateral aspect of the right upper and lower lids. The right side of his face had been scalded with oil 6 years previously. Biopsy showed a flat type of squamous papilloma with occasional atypical cells and mitoses, but no evidence of frank malignancy. The lesion was, therefore, treated by diathermy under local anaesthesia. In July 1964 he had a recurrence of the keratotic condition of the right upper and lower lids; a second biopsy showed no evidence of malignancy, but because of the sinister clinical appearance, both lids were excised and reconstructed. The excised tissue contained a well differentiated squamous cell carcinoma. In January 1966 he had a recurrent warty lesion at the right outer canthus extending on to upper and lower lids, and an enlarged lymph node in the right pre-auricular area. Biopsy of the lesion showed an anaplastic squamous cell carcinoma, a very different histological picture from that of 1964, but biopsy of the lymph node showed no evidence of squamous cell carcinoma. Because of conjunctival involvement the right eye was enucleated and post-operative radiotherapy was given to the pre-auricular nodes. Further recurrence developed after initial improve-

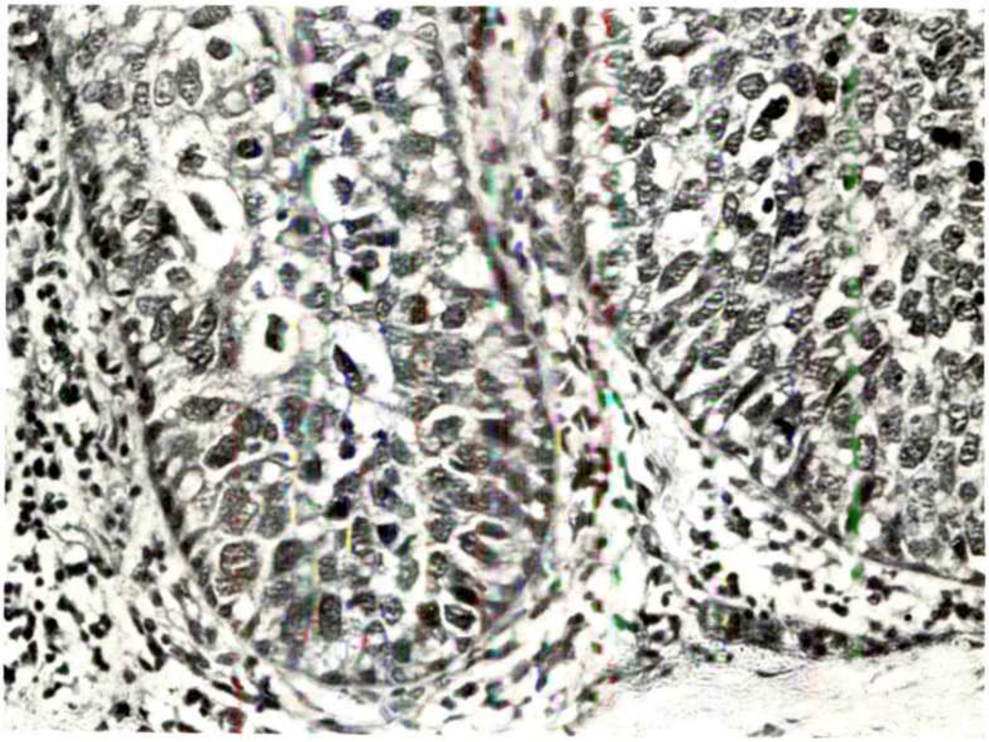

FIGURE 5. High power view showing malignant cells lining sebaceous glands of the eyelids (Case 3). (Haematoxylin and Eosin × 400.)

ment, and in February 1967 the patient died of secondary carcinomatosis from the primary squamous cell carcinoma on the right outer canthus. It seems probable that the initial biopsies in this case had been inadequate to reveal the true histopathological picture, as the affected follicles were not included in the rather superficial specimen.

COMMENT

(a) *Incidence*

This group of twenty-two cases of intra-epidermal carcinoma of the eyelid margin forms approximately 6·1% of all the malignant tumours of the eyelid referred to the Combined Clinic from 1959 to 1973.

(b) *Duration*

The duration of the tumours prior to referral varied from a matter of a few weeks in two cases to 10 years in one.

(c) *Age of patients*

Table 3 shows the age incidence for the period 1959–1973 of:

(1) intra-epidermal carcinoma of the eyelid margin,

(2) intra-epidermal carcinoma of all other sites,

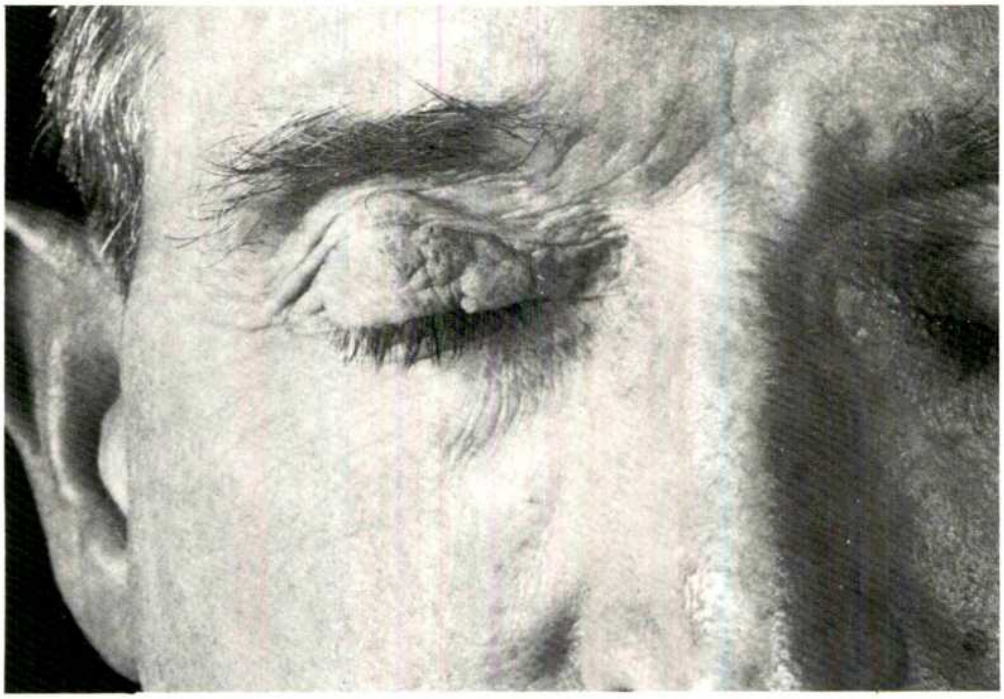

FIGURE 6. Case 4 showing intra-epidermal carcinoma of R. upper lid of 10 years' duration.

TABLE 3. Age incidence of tumours of eyelids and skin

Age	I.E.C. of eyelid margin			I.E.C. all areas			S.C.C. all areas		
	Male cases	Female cases	%	Male cases	Female cases	%	Male cases	Female cases	%
Not recorded	—	—	—	2	—	0·5	1	1	0·3
10+	—	—	—	—	—	—	2	—	0·3
20+	—	—	—	—	1	0·25	1	—	0·15
30+	1	—	4.5	3	3	1·5	5	4	1·4
40+	1	2	13·7	16	17	8·4	43	7	7·55
50+	5	1	27·3	39	35	18.8	104	17	18·3
60+	9	—	40·9	91	54	36·9	140	52	29·1
70+	3	—	13·6	68	64	33·65	184	99	42·9
Total	19	3	100·0	219	174	100·0	480	180	100·0

(3) squamous cell carcinoma of all sites.
These figures indicate that intra-epidermal carcinoma of the eyelid margin occurs at a somewhat earlier age than it does on other sites.

(d) *Site of lesions*
Two patients had an intra-epidermal carcinoma in the vicinity of the outer canthus with involvement

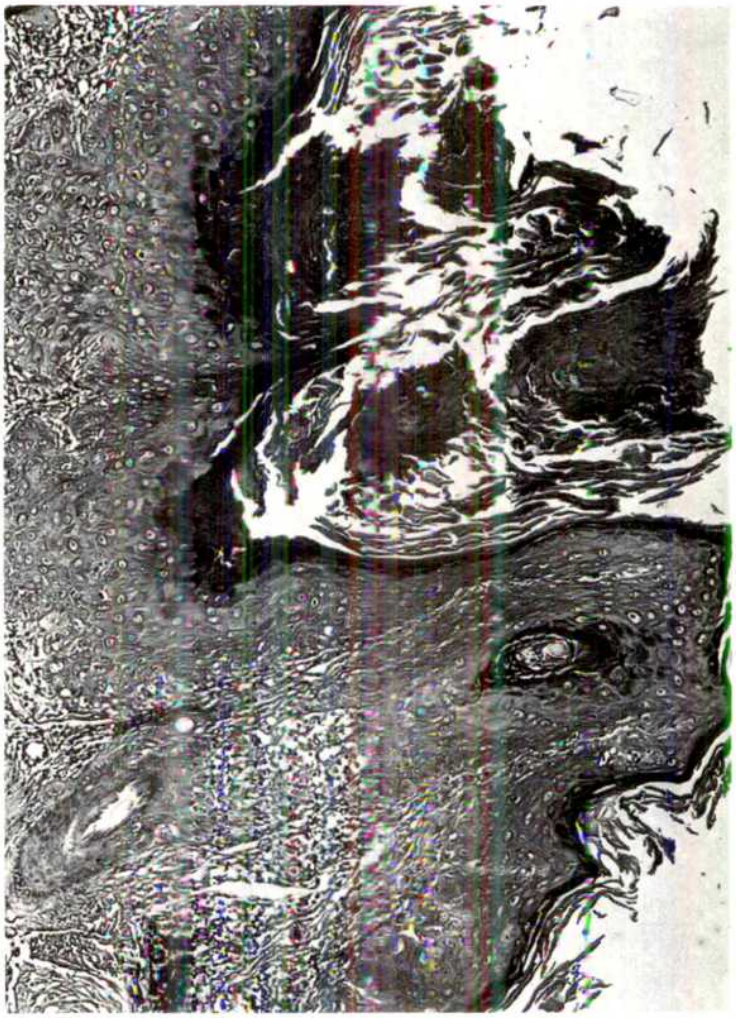

FIGURE 7. Low power view of warty type of intra-epidermal carcinoma of eyelid margin. There is marked hyperkeratosis and acanthosis with rounded downgrowths into the inflamed dermis (Case 4). (Haematoxylin and Eosin ×40.)

of both upper and lower lid margins. The lid involvement, with the inclusion of these two, was as follows:

Right upper, 7; Right lower, 6; Left upper, 7; Left lower, 4.

(e) *Occupation*

Fourteen of the twenty-two patients had done work which entailed handling oils and greases (Table 2). One of the males who was not engaged in work of this sort had had a basal cell carcinoma in the vicinity treated with X-rays 25 years previously. Of the three females, two were housewives and one a shorthand-typist.

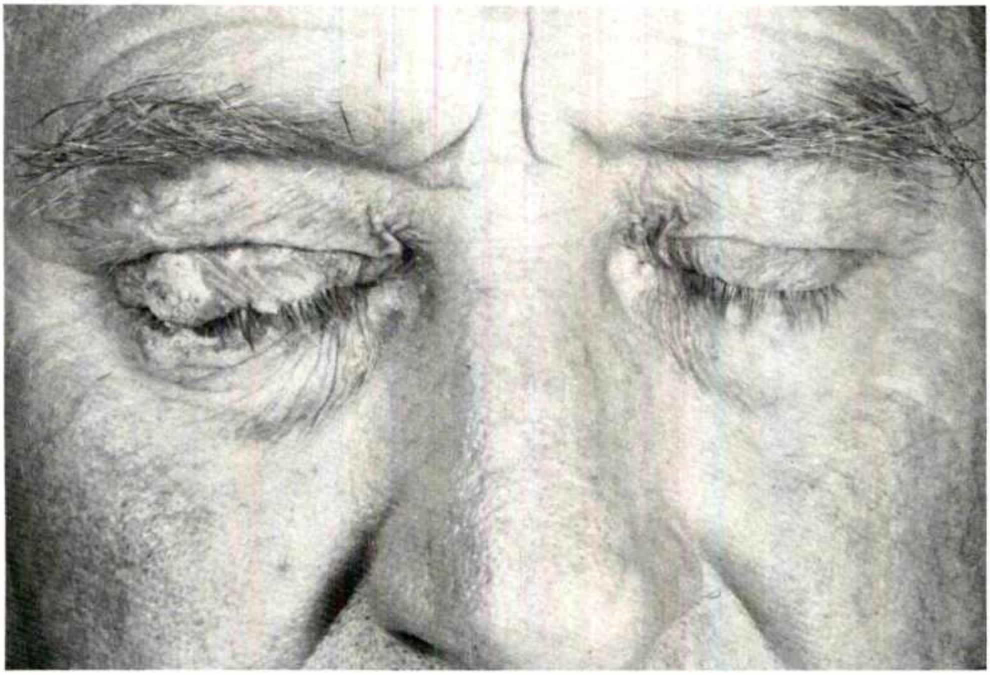

FIGURE 8. Case 5, extent of involvement in 1964.

(f) *Colour*

No note was made of the skin and hair colour of these patients, except that all were Caucasians, but in any future investigation of this disorder colouration should be recorded.

(g) *Clinical course of the disease*

The clinical appearance in the early stage was that of a benign keratosis or warty lesion of the lid margin. In long standing cases the lesions tended to develop horny plugs close to the lid margin which then became nodular, presumably due to extension of the intra-epidermal carcinomatous change into the lash follicles. This downgrowth may in due course destroy the follicle and its eyelash. In three of the twenty-two patients the intra-epidermal carcinoma had penetrated the epidermal barrier and given rise to an invasive squamous cell carcinoma, which proved fatal in the fifth case referred to above. Three of the non-invasive lesions were inadequately excised initially, and had to be retreated surgically. Because of the involvement of the lash follicles by the intra-epidermal carcinoma, superficial treatment will fail, resulting in loss of time and so increasing the possibility of invasion of the dermis by squamous cell carcinoma.

Histopathology

All twenty-two cases showed features of intra-epidermal carcinoma, but with a variable degree of dedifferentiation and cytological atypicality. At the extremes of the spectrum two main types of lesion are identifiable.

(1) A hyperkeratotic, warty, papillary lesion with marked acanthosis and bulky rounded downgrowths into the mildly inflamed dermis (Fig. 7). The basal layer is clearly defined, unless frank

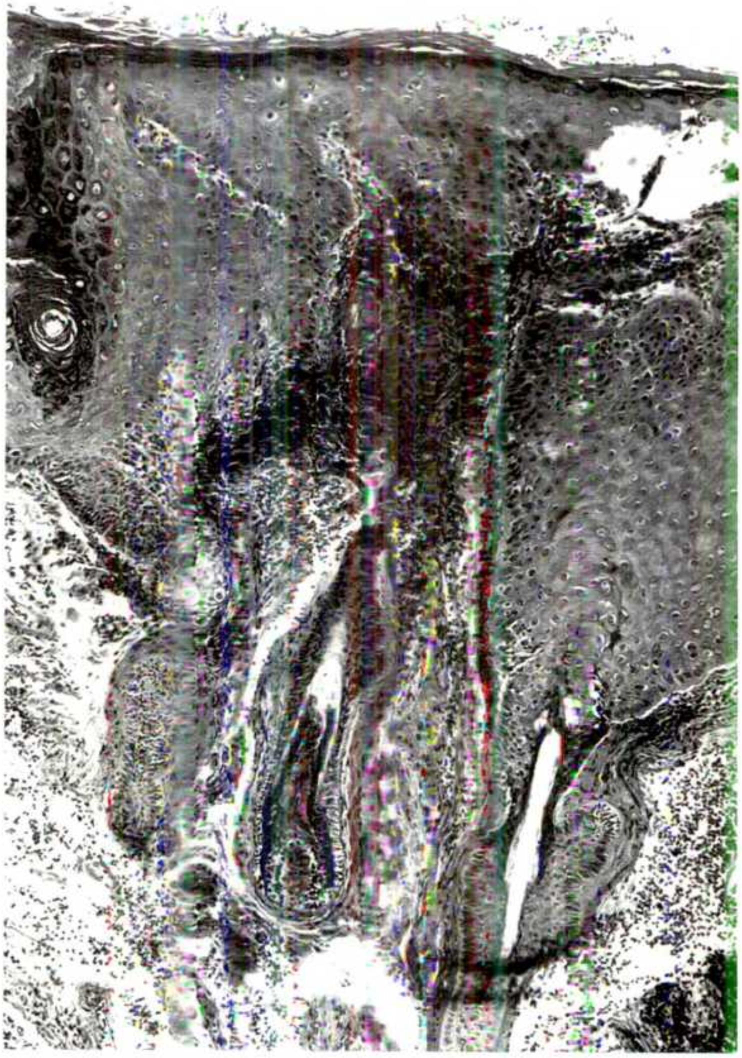

FIGURE 9. Low power showing marked acanthosis and rete peg prolongation with growth along the lash follicle. (Haematoxylin and Eosin × 40.)

invasive changes occur. There is only slight loss of polarity, and the proliferation is mostly of regular appearance, but mitotic figures and individual cell keratinization can be found on careful search. The superficial part of the tumour can appear so regular in its maturation and keratinization that an inadequate biopsy may give a false impression of innocence, as in the first two biopsies in Case 5 (Fig. 9) Even when the complete lesion is available for examination, misinterpretation of the appearances can readily occur, unless the potential of the type of lesion on this site is appreciated.

(2) A non-keratinizing or slightly keratotic lesion of flatter contour and usually with a slightly scaly surface. The histological appearances conform to the more classical descriptions of intra-epidermal carcinoma in other cutaneous or mucosal sites. Cellularity, pleomorphism, mitotic activity and loss of polarity are obvious and individually keratinizing cells are readily seen (Fig. 4). Downgrowths of

rete pegs are more extensive and pointed than with the keratinizing lesions, and the basal layer is commonly obscured by a dense dermal inflammatory cell infiltrate. This type appears to grow more rapidly and spread more extensively than its keratotic counterpart.

It should be appreciated that several of the cases in this series showed a histological appearance intermediate between the two types identified above. All these lesions are liable to extend along the ducts of appendage structures (Figs. 4 and 5), and, as a result of the peculiar depth and position of the lash follicles (Fig. 1), growth in this direction may well be much greater than can be clinically assessed with accuracy. Sections cut parallel to the lid margin demonstrate involvement of the adnexae much more satisfactorily than sections taken at right angles to the edge (Fig. 5, Table 2, Case 3).

Treatment

As intra-epidermal carcinoma invades the ciliary adnexae, in advanced cases to their deepest element, and because of the size of these adnexae in the eyelids, when the diagnosis has been established by adequate biopsy, we submit that surgical excision is mandatory. The tumour must be ablated; it is often difficult to assess whether this has been achieved in depth, especially where the skin and adnexae are involved. Complete excision must be achieved regardless of the need to preserve any particular structure; the surgeon must, therefore, be prepared to do reconstructive surgery. Fortunately excision and direct suture of the skin to the conjunctiva can usually be achieved, but the pathologist must state whether he considers that removal is complete in all directions; if the excision is inadequate further surgical procedures will be necessary. A description of most of these procedures form alignant tumours of the eyelids was published by Shulman (1962).

The following procedures were carried out in the present series:

(1) *Excision of the ciliary margin with direct closure*

This was performed on ten patients, including case No. 1 above, either by a wedge excision with direct closure or by removal of the whole of the ciliary margin by a longitudinal incision followed by advancement of the skin upwards or downwards as in an eyelid reduction.

(2) *Free Grafting*

Six patients. For lesions covering a large area of skin without involving the conjunctiva, the whole of the eyelid skin and ciliary margin were excised and the defect covered by a free graft. For the upper lid a thin split-graft from the arm is required, and for the lower lid a full thickness post-auricular graft.

(3) *Medial advancement flap of either eyelid*

This procedure is suitable for the removal of tumours which involve up to one half of either eyelid and which do not affect the conjunctiva; it was considered necessary in two patients.

(a) After excision of the lesion the lid margin is divided leaving the conjunctiva intact and carried laterally on to the temporal area. A further incision is then made from the lowest part of the defect, carried laterally along the lower border of the eyelid and continued in a slightly downward, oblique line on to the malar area. The skin, muscle and tarsal plate are then elevated in to the most lateral portion of the incisions, and advanced medially to fill the defect.

(b) Where it is found necessary to perform a full-thickness excision of the affected portion of the eyelid, the incisions are made slightly differently. Firstly, an incision extends from the external canthus laterally on to the temporal skin for approximately 2·5–4 cm. The lower incision extends from the inferior edge of the excision laterally along the lower edge of the eyelid, and is extended for approximately 5 cm on to the malar region, in a slightly oblique downward direction. The conjunctiva at the lower fornix is divided, and the lateral portion of the eyelid freed completely, leaving it based on the lateral pedicle. The conjunctiva at the lateral edge of the sclera is then divided leaving the conjunctiva attached to the flap. The whole flap is then advanced medially allowing the cornea to be covered by an island of conjunctiva. After suture of the flap, the conjunctiva regenerates at the lateral margin, leaving minimal deformity. This operation results in the loss of some eyelashes at the outer part of the lid.

(4) *Total removal of eyelid: upper or lower*

Three patients. When an eyelid, either upper or lower, is totally involved, complete removal is required with some of the peri-orbital fat. The conjunctiva is left flush with the upper or lower edge of the sclera, with the loss of the fornix. It is possible to reconstruct the eyelid by a modification of Henry's operation. The conjunctiva of the remaining eyelid is carefully dissected from the tarsal plate to the full extent of the fornix. The tarsal plate may be included in the dissection. Either procedure ensures that there will be sufficient conjunctiva to cover the exposed globe when sutured to the free scleral margin with 6/0 catgut. The excised skin is replaced by a free graft of split skin from the inner aspect of the arm for the upper lid and by a post auricular Wolfe graft for the lower. A total tarsorrhaphy is then performed between the graft and the remaining eyelid, and maintained for 3–6 weeks until the graft is soft, before division. The conjunctiva and skin are then sutured with 5/0 silk.

(5) *Exenteration of the orbit*

One patient. This radical and mutilating procedure was performed only where the disease had advanced beyond the limits of primary excision. In this case histological examination showed that excision had been incomplete and the tumour had invaded muscle. A prosthesis was required for the resultant deformity.

ACKNOWLEDGMENTS

We are grateful to our colleagues in the Combined Clinic for their co-operation; to Mrs J.Jones and Mrs M.Andrews for invaluable secretarial help; and to Mr E.Macleod and his staff for the photography.

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Is the cyclic AMP in psoriatic epidermis low?*

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Accepted for publication 9 December 1974

SUMMARY

A deficiency of epidermal cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) has been implicated as the cause of the incomplete differentiation, increased mitosis and increased glycogen concentration which characterize the psoriatic lesion. This communication contains our data on the epidermal concentration of cyclic AMP in the lesional and 'normal' (non-involved) skin of patients with psoriasis. In these studies we have introduced the following improvement in methodology: (1) Local ischaemia in the biopsy specimens was prevented by not giving any injections prior to removal of the tissues and by freezing the tissues prior to removal. (2) The epidermis was microdissected from the dermis prior to cyclic AMP assay so as to give as pure an epidermal specimen as possible.

Epidermal cyclic AMP was found to be 20–25% higher in the skin lesion than in the uninvolved skin when compared on a dry weight or protein basis but essentially the same when compared on the basis of DNA content. Our conclusion is that the cyclic AMP level in the psoriatic lesion is not lower than that in the 'normal' skin and thus these characteristics in psoriatic lesions cannot be related simply to a cyclic AMP deficiency.

The prominent pathological features of the psoriatic lesion are incomplete differentiation, increased cell proliferation, and glycogen accumulation. Voorhees & Duell (1971) ascribed these findings to a possible lack of cyclic AMP in the lesion. Cyclic AMP is known to suppress mitosis in epidermal cells (Bullough & Laurence, 1964, Voorhees *et al.*, 1972a) and to decrease glycogen in various organs by simultaneously activating phosphorylase and inhibiting glycogen synthetase (Robison, Butcher & Sutherland, 1971). The theory, therefore, explained many things in a reasonable fashion and had great appeal. Subsequent studies by Voorhees and his associates (1972b; 1973a,b) indicated that the epidermis in psoriatic lesions did indeed contain lesser amounts of cyclic AMP than did the epidermis of the uninvolved skin.

These measurements were done on keratome strips of skin removed after the local injection of an anaesthetic. Recently Härkönen, Hopsu-Havu & Raij (1974) measuring cyclic AMP in similar strips

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* Supported in part by Grant AM 17179 of National Institutes of Health.

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of skin reported that the lesion contained a higher concentration than the uninvolved skin. Although the techniques used to measure the cyclic AMP were different, this should not account for the opposite conclusions reached by the two groups.

We have also measured cyclic AMP in the involved and uninvolved epidermis of psoriasis patients. Unlike the preceding investigations these measurements were carried out on tissues which had not been subjected to ischaemia which increases the cyclic AMP level (Yoshikawa *et al.*, 1975b), and consisted as far as possible (95%) of pure epidermis. Our results are in agreement with the findings of Härkönen *et al.*, i.e. the cyclic AMP level in the psoriatic lesion is not decreased and in fact may be slightly increased.

MATERIALS AND METHODS

Biopsies were taken from ten male adult patients with psoriasis. The patients had received neither topical nor systemic treatment for 3 weeks prior to biopsy. Local injection of anaesthetics before biopsy was avoided. Local cleaning of the skin surface was also avoided since it has been reported that rubbing of the skin stimulates mitosis; thus the surface cleaning may change the endogenous level of cyclic AMP.

The psoriatic lesion and adjacent normal appearing skin were simultaneously frozen by an ethyl chloride spray. Biopsies were done by two persons, i.e. one continually sprayed so that the skin to be biopsied was kept frozen all the time, and the other removed two small cylinders of skin with a 6 mm punch attached to an electric motor. The involved lesion was always taken first, then the uninvolved.

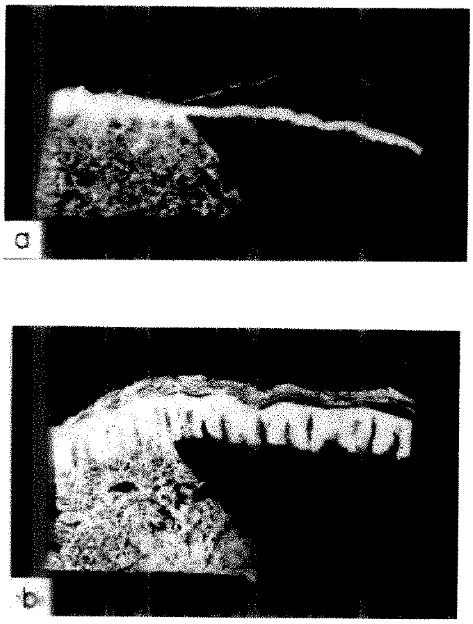

FIGURE 1. Microdissection of epidermis. Epidermal samples were dissected from frozen dried skin under a stereomicroscope. The keratin layer was completely peeled off. Separation of epidermis from dermis required microdissection. The papillary dermis between the epidermal rete ridges in the psoriatic lesion were carefully removed; thus the purity of the epidermis is approximately 95%. (a) The uninvolved and (b) The involved epidermis from a psoriatic patient.

The distance between the two biopsies was 3–4 cm. The cylinder of skin biopsied was cut vertically (perpendicular to the skin surface) at 20 μm thickness at −30°C in a cryostat, and the sections were freeze-dried under vacuum for 16–24 h. The samples were not allowed to thaw during all these procedures.

These thin freeze-dried sections showed a clear epidermal–dermal junction without staining. Epidermal samples were microdissected under a stereomicroscope by free hand (Fig. 1). For cyclic AMP measurement, 50–100 μg of 'pure' epidermis was weighed with a Cahn RG electric balance and placed in a home-made micro test tube, 2·5 × 35 mm, for the analysis. Cyclic AMP was measured in quadruplicate or quintuplicate by the protein binding method (Gilman, 1970) as modified by Yoshikawa *et al.* (1975a). For DNA determination, 7–15 μg of 'pure' epidermis was used in triplicates or quadruplicates (Kissane & Robins, 1958; Santoianni & Ayala, 1965). For protein measurement 50 μg of pure epidermis was used after NaOH digestion (Lowry *et al.*, 1951).

RESULTS

The cyclic AMP levels in the uninvolved and involved epidermis from ten psoriatic patients are summarized in Table 1. On a dry tissue weight basis, the average cyclic AMP level in epidermis from normal looking skin was 3·01 pmol/mg, in the psoriatic lesion, 3·83. The result of a paired *t*-test indicates that this difference is significant at the level of $0{\cdot}01 > P > 0{\cdot}005$ (n = 10). When the cyclic AMP level was compared on a tissue protein basis in psoriatic (involved) epidermis, it was 20% higher than in uninvolved epidermis; the statistical significance was similar ($0{\cdot}01 > P > 0{\cdot}005$, n = 10). However, when the cyclic AMP level was compared on a DNA basis, no significant difference between the uninvolved and involved epidermis from psoriatic patients was observed ($P > 0{\cdot}7$, n = 10). Whether this slightly increased level of cyclic AMP in psoriatic epidermis has any biological significance is still not clear, but the difference on a weight or protein basis is statistically significant. The obvious

TABLE 1. Epidermal cyclic AMP levels in psoriasis

	Uninvolved			Involved		
	cAMP (pmol)			cAMP (pmol)		
Case no.	mg dry tissue	mg prot.	μg DNA	mg dry tissue	mg prot.	μg DNA
1	1·59	2·11	0·096	2·89	3·71	0·096
2	2·77	3·68	0·286	3·08	4·01	0·101
3	1·96	2·50	0·087	2·87	3·61	0·179
4	1·68	2·17	0·090	2·72	3·51	0·162
5	4·92	6·42	0·190	5·33	6·50	0·282
6	6·35	8·62	0·415	6·04	8·15	0·276
7	1·63	2·16	0·094	2·15	2·81	0·110
8	2·80	3·95	0·172	3·61	4·87	0·242
9	2·87	3·54	0·179	5·17	5·88	0·215
10	3·48	4·01	0·247	4·44	5·67	0·298
Average	3·01	3·92	0·186	3·83	4·87	0·196
± s.e.	± 0·49	± 0·67	± 0·034	± 0·42	± 0·53	± 0·025

conclusion is that, contrary to Voorhees and associates' report (1972b; 1973a,b), the cyclic AMP level in the involved epidermis is *not lower* than that in the uninvolved epidermis.

DISCUSSION

Modifications in the way the tissues were treated prior to removal were made because just as in brain Kakiuchi & Rall, 1968) ischaemia of the skin results in elevation of the cyclic AMP level. Cyclic AMP can increase two to three-fold within 10–20 s (Yoshikawa *et al.*, 1975b). This may explain the fact that both Voorhees and Härkönen's data are higher than ours in spite of dermal contamination.

TABLE 2. Cyclic AMP levels in psoriasis in the literature

	Uninvolved			Involved		
	cAMP (pmol)			cAMP (pmol)		
Reference	mg wet tissue	mg prot.	μg DNA	mg wet tissue	mg prot.	μg DNA
Voorhees *et al.* (1972)	1·4	9·1	0·7	0·9	4·1	0·4
Voorhees *et al.* (1973)	1·2	12·3	1·1	1·0	7·0	0·7
Härkönen *et al.* (1974)	1·62	—	—	2·0	—	—
Our data	0·91 (3·01/3·3)	3·92	0·186	1·16 (3·83/3·3)	4·87	0·196

Table 2 summarizes the data on cyclic AMP in psoriatic epidermis. Our data have been converted for the sake of comparison from the original dry weight basis to a wet weight basis using the ratio of dry to wet weight of 1:3 (Stankler, 1969). The methods as stated before differ for the various measurements: Voorhees' 1972 data were obtained by the method of Brooker, Thomas & Appleman (1968); Härkönen *et al.* used the radioimmunoassay of Steiner *et al.* (1969) while Voorhees' 1973 data and ours were based on the protein binding assay of Gilman (1970). It is noteworthy that the results are closest on a tissue weight basis and become more divergent in relation to tissue protein and especially DNA. In fact Voorhees' 1973 data were not significantly different (1·2 vs 1·0 pmol) on a weight basis. Because of this we looked more closely at the reason for the great differences in the results expressed by protein and DNA bases.

Table 3 shows the values for DNA content of the tissues as calculated from the respective data in addition to the data reported for epidermal DNA by Santoianni *et al.* (1963, 1965) and DeBersaque (1966). It is obvious that the DNA values reported by Voorhees were too low. Possible reasons could be a failure to extract all the DNA from the tissue, or a large dermal contamination. As shown in Table 4 the same problem exists but to much less extent in the protein values. This renders comparison on the basis of protein or DNA meaningless and the only comparison possible is on a weight basis. Since Voorhees' data, on this basis, also failed to confirm a definite decrease, we believe all of the studies are in agreement that cyclic AMP is not decreased in the lesion.

Our finding that the cyclic AMP level is not low in active psoriatic epidermis does not necessarily imply that it is not low in the basal cells. If it is low only in the basal cells of psoriatic epidermis, it

TABLE 3. DNA content of epidermis

Normal	Psoriatic		References
	Uninvolved	Involved	
0·44	—	—	Santoianni & Rothman (1963)
0·36	—	—	Santoianni & Ayala (1965)
0·39	—	0·44	De Bersaques (1966)
—	0·32	0·35	Voorhees *et al.* (1972)
0·28*	0·20*	0·23*	
—	0·11†	0·14†	Voorhees *et al.* (1973)
—	0·043‡	0·047‡	
—	0·52 (1·72/3·3)	0·63 (2·08/3·3)	This paper

DNA contents are expressed as g DNA/100 g wet weight tissue (%). Data by Santoianni, DeBersaques & ours were originally expressed on a dry weight basis. These are converted to a wet weight basis using a dry/wet weight ratio of 1:3.

Average DNA content of the uninvolved and the involved epidermis were reported by Voorhees *et al.* (1972) as 0·32 and 0·35% respectively but computation from the actual data in the same paper yielded 0·20 and 0·23% respectively (*).

† Computed from data on cyclic AMP levels in epidermis.

‡ Computed from data on cyclic GMP levels in epidermis.

TABLE 4. Protein content of epidermis

Normal	Psoriatic		References
	Uninvolved	Involved	
	16·4	20·1	Voorhees *et al.* (1972)
15·6*	15·4*	22·0*	
	9·8†	14·3†	Voorhees *et al.* (1973)
	2·0‡	2·0‡	
	23·4 (77·1/3·3)	24·8 (78·6/3·3)	This paper

Protein content is expressed as g protein/100 g wet tissue weight (%).

* Computed from data on cyclic AMP levels in epidermis in the same paper by Voorhees *et al.* (1972).

† Computed from data on cyclic AMP levels in the paper by Voorhees *et al.* (1973).

‡ Computed from data on cyclic GMP levels from the same paper.

must be relatively much higher in the prickle and granular layers to give an average which is higher than normal. If this was the case, another problem would be posed, namely, why the cyclic AMP level is higher in the prickle layer where the glycogen content is markedly increased. If the triad that accompanies psoriatic lesions—incomplete differentiation, accumulation of glycogen, and accelerated mitosis—is related to cyclic AMP deficiency, the whole epidermis should share the responsibility, since the major site of differentiation is at the granular layer, that of glycogen accumulation at the prickle layer, and that of mitosis at the basal layer. Since the cyclic AMP level in whole psoriatic epidermis is not low, this triad cannot be related simply to a cyclic AMP deficiency. The biopathological control of the psoriatic epidermis appears to be complex and needs further study.

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Survey of skin disorders in the urban black population of South Africa*

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Accepted for publication 11 November 1974

SUMMARY

Numerous and different methods of survey have been suggested and developed in recent years, but none of them appears to be entirely satisfactory. Insufficient classification, inadequate follow-up, incongruous rotation of investigators, notable variations in attendance, racial differences of the population submitted to statistical study, and other minor factors may account for possible errors and differences in evaluating the findings.

We have conducted a survey, over a 3-year period, of the incidence of skin diseases affecting our *urban black population*, living in the township of Soweto (800,000 inhabitants) and attending our dermatological service at Baragwanath Hospital. Although this population is made up of a dozen different ethnic groups, they are fully urbanized and live under similar conditions and according to similar customs. We believe that the statistical data derived from these patients represent a significant expression of a uniform community.

The black population of South Africa has been the subject of many investigations in various medical disciplines. From these studies it has been possible to establish the anatomical, physiological and functional characteristics of this racial group.

Trowell (1960) summarized these features as follows:

(i) Low blood sugar (average 75 mg ± 19·1 mg%), low serum albumin, low urinary 17-ketosteroids, and low serum cholesterol and uric acid.

(ii) Increased urinary and plasma oestrogen and, as is obvious, increase of the tissue melanin, detectable also in circulating blood leukocytes.

(iii) Increased reticulo-endothelial-system activity counteracting the chronic stimulation by bacterial, parasitic and viral agents (Wassermann, 1972).

In dermatology some detailed research has been performed on patho-physiological aspects, and most of the findings of Clarke (1959) apply to our black population. These findings show that the African skin contains more sweat and sebaceous glands per unit area than does European. The Africans sweat more per unit area than Europeans for an equivalent effort. The dermis has a richer blood supply as a result of adaptation to a hot climate. In general the African's body hair is sparse with pepper-corn arrangement on the scalp. The African's skin shows darkest areas, the nipples and genitalia, in association with lighter areas in the supraorbital ridges, upper eyelids, malar prominences, over the dorsal spine, over the clavicles and down the middle line of the chest as far as the epigastrium. The palms and soles are pale, the nails dusky pink and may have longitudinal streaks of pigment. Mild injury limited to the epidermis results in an increase of pigmentation, whereas if the dermis is damaged with involvement or destruction of the basal layer a diminution or even a total absence of pigment may result.

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* This paper has been read at the III World Congress of Tropical Dermatology (São Paulo, September 1974) and awarded with Certificate of Honour.

Although some papers on the incidence of skin diseases of the black S. Africans have appeared in recent years (Findlay, 1957, 1967; Findlay & Park, 1969; Schultz, Findlay & Scott, 1962; Ross, 1966; Dogliotti, 1970; Marshall, 1972; Scott, 1972), the surveys and their classification were sometimes incomplete, and inadequate to offer real figures of frequency and distribution.

In the latest edition of the 'Textbook of Dermatology', Rook, Wilkinson & Ebling (1972) have remarked upon the gap in knowledge of skin diseases affecting not only the S. African blacks but also the entire African Negro community. The authors underline the necessity to revise the current data, and suggest a reassessment taking into account factors previously neglected—'there is no doubt that properly conducted surveys, critically interpreted, contribute greatly to our knowledge of the aetiology of many skin diseases'. Some of the literature has appeared in local publications, in various languages, and also in reviews not concerned solely with dermatological matters.

A recent census (1970) showed that the total population of blacks in Johannesburg accounts for 809,605 inhabitants. This community is composed of approximately a dozen different ethnic groups. The rapidly expanding industrial and mining populations have attracted an ever-increasing number of migrant labourers from the neighbouring states (Botswana, Lesotho, Malawi, Swaziland). These migrants easily adopt the customs and the way of life of resident urbanized Africans, living according to a similar life-style in a relatively homogenous city community. Therefore *we believe that the statistical data attached to it represent a significant expression of a uniform community*.

The resident blacks make up the large majority of the patients attending our service. From 7000 to 8000 persons are seen each year, and first consultations were 2300 in 1969 and 4500 in 1972. Of these patients 250–300 are admitted to the ward each year.

To complement our survey presented in 1969 (Dogliotti) we are in the position to show more detailed figures of incidence of skin diseases in our black population in this triennial survey from 1969 to 1972.

Ecology

Exogenous and endogenous factors affect morbidity, and Gillman (1970) stated that the most important of these may be: (a) genetic constitution, (b) climate, (c) diet and (d) hormonal relationships.

We consider these factors as the original cause of certain variations of incidence of cutaneous diseases, but others (Rook *et al.*, 1972) have stressed that there are additional elements that are equally important, namely social customs, working conditions, socio-economic standards, and available health facilities. These agents, seldom mentioned, have not received the attention that they deserve.

It is not our intention to discuss the genetic factors of S. African blacks whose details are reported elsewhere, but we will discuss more specifically the influence on skin disorders of other important agents mentioned by Gillman.

(i) *The climate* of Johannesburg where our population lives, the so-called *macroclimate* of Paul (1958), is temperate and has the following characteristics:

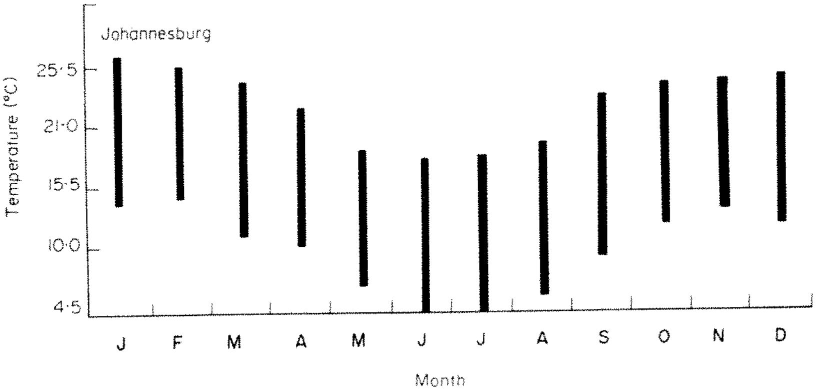


FIGURE 1.

Altitude—1753 m above sea level.
Mean temperature—21°C in summer and 10·5°C in winter (Fig. 1).
Mean duration of sunlight—8·7 h a day (Fig. 2).
Frequent summer rainfall characterized by extreme violence, short duration and electrical storms (Fig. 3).

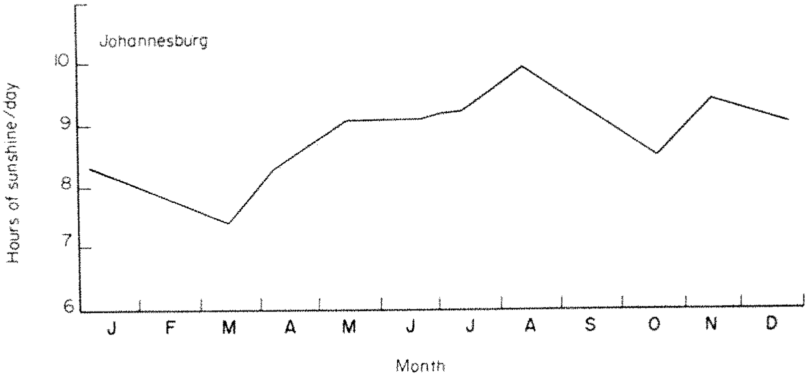


FIGURE 2.

Of paramount importance in determining the incidence of diseases is also the *microclimate* of Paul against which the individual has to defend himself. This is composed of socio-economic and domiciliary conditions (Canizares, 1972).

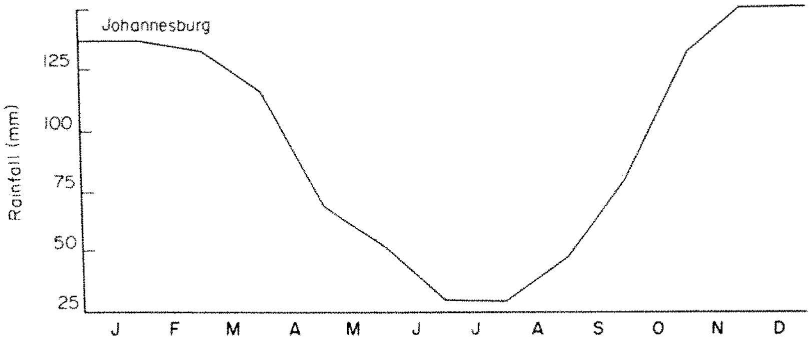


FIGURE 3.

It is important to emphasize the marked differences in the standard of living of the two groups of black Africans living in S. Africa:

The economically successful *urban* group, westernized from contact with the European community, rapidly developing and fundamentally emancipated from restrictions imposed by religious and ritualistic customs.

The *rural* group, from a conservative patriarchal regime, limited by ancestral prejudices and cultural and economic reactionism typical of all remote African communities.

(ii) *The nutritional status* of the urban blacks can be considered to be above that of Negro groups in the rest of Africa. It depends, of course, on the financial potential of different classes. The socio-economic standard amongst this population varies more widely than anywhere else in Africa. The staple diet of the entire population consists of a high intake of carbohydrates, more limited consumption of proteins and negligible fat consumption. Multiple deficiencies and a low protein/calories ratio have profound repercussions on nutritional status and morbidity, as does the high consumption of alcohol, often illegally manufactured.

(iii) *The conditions of work* vary inevitably in the two groups. The urban group exposed to the risks of modern industrialization is protected by legislation covering working hours, weekly and annual leave, medical assistance and transport. The medical and social facilities available to the rural community are more limited.

(iv) *Medical advice* is sought earlier by sophisticated people in towns than by their long-suffering rural counterparts, and there is, therefore, a tendency for more trivial conditions to be seen in the urban clinics. Snyman (1972) pointed out that geographical and educational considerations affect the accessibility of health facilities to the rural African. Nominal fees are charged to encourage patients to present at an early stage to urban hospitals in which preventive medicine is as important as curative medicine. Nevertheless, many of our patients have sought advice, both medical and paramedical, prior to attendance at our institution. This advice varies greatly in skill and competence. Ill-informed instructions may lead to delay in diagnosis and treatment of skin diseases, and iatrogenic complications from the misuse of drugs are frequent, notably with corticosteroids both systemic and topical. In spite of the availability of medical facilities, the traditional importance of the 'witch-doctor' has not been undermined. Although lacking in medical training in the accepted sense, he has a deep understanding of the psychology and social customs of his people. This enables him to become a more respected (and more wealthy) member of the community. With the support of witch-craft, religious practice and rituals such as initiation ceremonies, every form of therapy is employed for an unlimited variety of diseases. Not only does consulting a witch-doctor delay the commencement of correct medical treatment, but complications and side-effects arise, and the true nature of patients' cutaneous illnesses may be disguised. As well as herbal concoctions prescribed by native doctors, similar remedies are sold by herbalists and unqualified practitioners. These medicines, together with undesirable preparations available across the counter in the chemists shops, can have adverse effects upon the skin, as can cheap, poorly manufactured cosmetics.

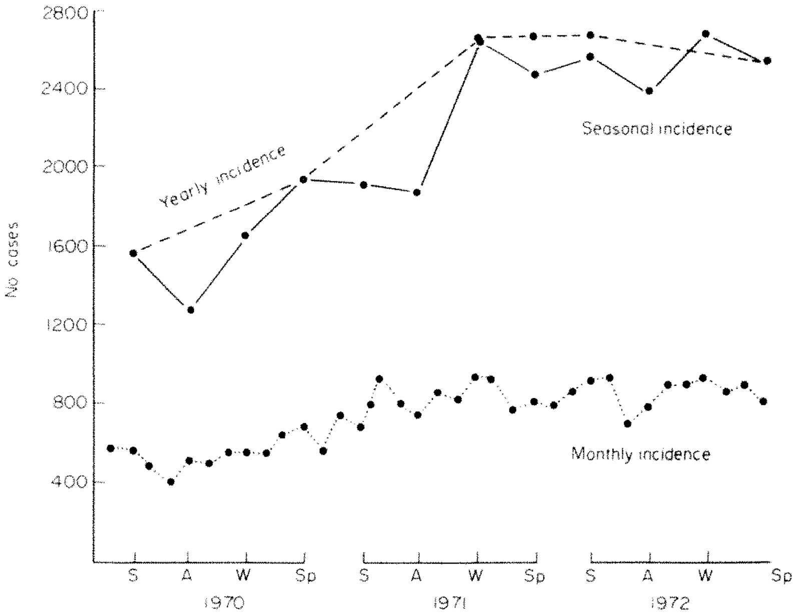
(v) *Contact with European civilisation* has encouraged the urban African to improve his standard of living and hygiene. His rural counterpart has not the same opportunity. The town-dweller is in a better position to make use of some of the results of western development. These include better education, more appropriate clothing, disinfection services, disposal of waste, better care of domestic animals, and ready availability of running water.

(vi) A factor hitherto insufficiently stressed is the *separation of the worker from his family* for long periods of time. In our experience this has an effect upon the incidence of dermatological and especially of genital infections. Many African labourers travel hundreds of miles from their homes, and the only opportunity they have to return to their families is their 1 month's compulsory leave. With the severing of his family ties, the labourer is exposed to the temptations and attractions of metropolitan life. New family units may be formed with obvious repercussions on the socio-economic conditions.

MATERIALS AND METHODS

We have conducted a statistical survey of cutaneous disorders affecting patients attending our consulting room at Baragwanath Hospital, employing as many parameters as we believed to be significant. Some authors (Rook *et al.*, 1972; Bannerjie & Datta, 1973) have pointed out that it is often less difficult to investigate hospital attendances than the population at large. In full agreement with this view, we have surveyed the incidence of the skin and genital diseases in our own hospital African urban population. We have concentrated on the following parameters:

(i) Total incidence of first and subsequent consultations.
(ii) Monthly incidence (Fig. 4).
(iii) Annual incidence (Fig. 4).
(iv) Seasonal incidence (Fig. 4).



Total attendance 25,841, New patients 9474

S, Summer, A, Autumn; W, Winter, Sp, Spring

FIGURE 4.

(v) Distribution of the diseases according to age and sex (Table 1).
(vi) Mean percentage of all disorders in relation to the total incidence.

TABLE 1. Skin disorders in the urban black population

Disease	Number of patients by age and sex																								Total	%	M/F ratio
Age	0/10			11/20			21/30			31/40			41/50			51/60			61/70			+70					
Sex	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%			
Bact. inf.	169	149	15·1	188	238	28·1	132	79	13·9	152	92	16·1	99	54	10·1	58	27	5·60	20	20	2·6	21	14	2·3	1512	15·9	1·85:1·4
Acne	—	—	—	74	83	57·9	54	46	40·6	1	2	1·1	—	1	0·4	—	—	—	—	—	—	—	—	—	271	2·9	1·28:1·2
Albinism	4	5	52·9	2	—	11·8	1	1	11·8	1	1	11·8	1	1	11·8	—	—	—	—	—	—	—	—	—	17	0·2	1·10:1·0
Alopecia	—	1	3·3	3	8	36·7	11	3	46·7	1	3	13·3	—	—	—	—	—	—	—	—	—	—	—	—	30	0·3	1·00:1·0
Atopy	3	7	50·0	1	5	30·0	2	—	10·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	20	0·2	0·80:1·2
Callus	1	7	4·1	14	13	13·9	22	12	17·6	26	14	20·6	19	12	16·0	18	12	15·5	13	11	12·4	—	—	—	194	3·9	1·39:0·7
Cheilitis	1	—	6·2	—	—	—	3	1	25·0	1	—	6·2	3	1	25·0	2	—	12·5	—	4	25·0	—	—	—	16	0·2	1·00:0·6
Cond. acum.	8	16	5·60	108	92	46·0	85	40	29·2	21	12	7·7	11	13	5·6	11	11	5·1	—	—	—	—	—	—	428	4·5	1·32:0·7
Cont. derm.	53	53	11·6	67	141	22·6	62	152	23·3	44	115	17·6	49	76	13·6	31	42	8·0	18	16	3·7	7	—	0·1	920	9·7	0·54:1·8
Derm. herp.	1	3	28·6	1	2	21·4	—	2	14·3	1	1	14·3	—	1	7·1	2	—	14·3	—	—	—	—	—	—	14	0·1	0·55:1·8
D.I.E	—	2	3·0	—	2	3·0	5	18	34·8	2	18	30·3	—	12	18·2	2	4	9·1	—	1	1·5	—	—	—	66	0·7	0·11:6·3
Drug erup.	4	2	5·2	11	9	17·2	10	26	31·0	13	8	18·1	11	12	19·8	3	2	4·3	2	1	2·6	1	1	1·7	116	1·2	1·10:1·2
Eczema	128	114	19·6	54	82	11·1	98	95	15·6	125	80	16·6	127	60	15·1	77	41	9·6	63	47	8·9	21	21	3·4	1233	13·0	1·30:0·9
Eryt. ind.	—	—	—	—	1	5·9	—	5	29·4	—	8	47·0	2	—	11·8			—	—	—	—	—	1	5·9	17	0·2	1·00:8·0
Eryt. mult	10	6	20·25	9	11	25·3	12	11	29·1	5	5	12·6	6	2	10·1	—	1	1·3	—	1	1·3	—	—	—	79	0·83	1·00:0·9
Eryt. nod.	—	1	3·6	—	12	42·8	2	4	21·4	—	5	17·8	—	2	7·14	—	1	3·6	—	—	—	—	1	3·6	28	0·3	1·00:12·0
Erythrod.	—	—	—	—	—	—	3	—	12·0	2	1	12·0	6	1	28·00	3	1	16·0	3	1	16·0	3	—	16·0	25	0·3	5·00:1·0
Mycot. sup.	68	71	24·6	47	48	16·8	72	54	22·3	40	44	14·8	27	35	10·9	16	15	5·4	11	16	4·7	—	—	—	564	5·9	1·05:1·1
Mycot. deep	—	—	—	—	3	11·1	3	2	18·5	5	2	25·9	2	3	18·5	4	1	18·5	1	—	3·7	1	—	3·7	27	0·3	1·00:0·6
Gonorrhoea	—	—	—	83	11	25·1	193	11	54·5	37	7	17·1	11	—	2·9	1	—	0·2	—	—	—	—	—	—	374	3·9	12·80:0·7
Herpes S.	3	6	9·5	14	16	31·9	21	14	37·2	4	7	11·7	5	2	7·40	1	1	2·1	—	—	—	—	—	—	94	0·9	1·20:1·1
H. zoster	1	1	2·9	5	9	20·5	8	9	25·0	5	4	13·20	4	1	7·3	7	3	14·7	9	1	14·7	—	1	1·4	68	0·7	1·19:1·4
Ichthyosis	4	2	60·0	1	1	20·0	—	—	—	—	—	—	1	—	10·0	1	—	10·0	—	—	—	—	—	—	10	0·1	2·50:1·0
Leprosy lep	—	1	4·1	—	—	—	2	1	12·5	4	1	20·8	7	3	41·8	2	—	8·3	3	—	12·50	—	—	—	24	0·25	300:100
Leprosy tub.	—	—	—	—	—	—	—	1	9·1	1	1	18·1	4	—	36·3	1	1	18·1	1	—	9·1	—	1	9·1	11	0·1	1·70:1·0
Lichen pl.	2	1	4·0	4	11	20·0	5	11	21·6	7	9	21·6	7	4	17·50	5	4	12·1	1	1	2·7	—	—	—	74	0·8	1·00:1·4
Lichen urt.	8	16	88·8	1	2	11·2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	27	0·3	1·00:2·0
Lupus vulg.	3	3	31·5	—	3	21·0	—	1	5·2	—	3	15·7	1	1	10·5	2	—	10·5	—	—	—	1	—	5·2	19	0·2	1·10:2·0
Moll. cont.	5	8	61·1	—	3	14·2	4	—	19·0	—	—	—	—	—	—	x	—	4·7	—	—	—	—	—	—	21	0·2	1·00:1·1
Neuroderm.	—	—	—	2	3	13·5	—	4	10·8	2	4	16·2	9	2	29·7	3	2	13·5	3	3	16·2	—	—	—	37	0·4	1·00:1·0
Neurofibr.	—	—	—	—	—	—	3	1	36·3	—	1	9·1	1	3	36·30	—	—	—	2	—	18·1	—	—	—	11	0·11	1·00:0·8
Pellagra	1	1	1·1	1	3	2·1	28	25	28·1	40	33	38·8	31	10	21·8	9	3	6·4	2	—	1·1	1	—	0·5	188	2·0	1·13:0·7
Pemphig. v.	—	—	—	—	—	—	—	1	10·0	1	2	30·0	—	3	30·0	—	—	—	—	2	20·0	—	1	10·0	10	0·1	0·10:1·0
Pit. rosea	20	32	21·3	39	50	36·6	27	30	23·2	18	15	13·5	12	—	4·9	—	—	—	—	—	—	—	—	—	243	2·6	0·91:1·0
Pruritis	—	1	2·5	1	1	5·00	3	5	20·0	8	3	20·00	—	—	—	3	—	7·5	5	—	12·5	5	—	12·5	40	0·4	1·00:0·6
Psoriasis	2	6	5·0	8	21	18·8	21	17	23·9	23	11	21·3	19	12	19·4	6	5	6·9	4	1	3·1	1	1	1·2	159	1·7	1·50:0·9
Scabies	27	25	29·7	22	31	30·2	21	11	18·2	10	8	10·2	5	3	4·6	5	5	5·6	—	1	0·6	—	1	0·6	175	1·8	1·10:1·0
Syph. prim.	—	1	0·0	293	39	36·60	413	19	41·6	99	5	11·4	25	—	21·7	8	1	1·0	2	1	0·0	—	—	—	906	9·6	7·54:0·1
Syph. second.	4	7	0·12	103	354	53·8	121	117	28·00	47	35	9·6	21	19	4·7	19	—	2·23	1	—	0·0	1	—	0·0	849	9·0	0·92:1·0
Ulcer leg	—	—	—	1	2	4·6	5	10	20·8	8	11	26·3	7	8	20·8	9	6	20·8	3	2	6·9	—	—	—	72	0·8	1·00:1·3
Urticaria	9	10	9·4	27	35	30·6	13	38	25·2	16	24	19·8	8	10	8·2	6	5	5·4	1	—	0·5	—	—	—	202	2·1	0·80:1·22
Vitiligo	4	10	25·00	2	13	26·7	2	6	14·2	3	3	10·7	3	2	8·92	3	4	12·5	1	—	1·8	—	—	—	56	0·6	0·90:1·9
Warts	22	16	16·7	26	30	24·6	18	25	18·8	18	10	12·3	14	12	11·4	11	13	10·5	12	—	5·2	—	—	—	227	2·4	1·14:0·9

Survey from 1969 to 1972.
First consultations: 9474; total consultations: 25,841.
Grand total: 9474.

(vii) Percentage of monthly and annual incidence and mean percentage, related to the total incidence, of the more common conditions observed during the 3-year period 1969–1972.

(viii) In order to avoid diagnostic variations membership of our unit was constant during the entire period of this survey.

(ix) Particular attention was paid to recording nutritional status, medical details, the patient's past and family history, and notes on his social and occupational environment.

Cases presenting with difficult diagnostic problems were admitted to hospital for thorough assessment. Laboratory investigations were carried out with the collaboration of the experts of our institution (SAIMR).

Gonorrhoea and other genital infections were included in this survey because they are traditionally seen in our consultation room, and these conditions are included in the medical facilities granted to our population.

Our classification is based on that used in contemporary textbooks of dermatology and pathology, mainly Degos (1953), Fitzpatrick *et al.* (1971), Montgomery (1967), and Rook *et al.* (1972).

We have made certain simplifications in our classification: under the heading 'Bacterial Infections' we have included impetigo, furunculosis, ecythma and erysipelas, and also the secondary complications of pre-existing skin diseases. The superficial mycoses include various forms of tinea, pityriasis versicolor, muco–cutaneous candidiasis, and the variety of epidermophytosis localized in the skin folds; whereas the group of deep mycoses include sporotrichosis, maduromycosis, chromomycosis, and the rare cutaneous aspergillosis.

COMMENT

The figures of this survey show that the total attendance for 1972 was twice that for 1970, and it is difficult to offer a satisfactory explanation of this increase. The following may have been the causes of significant importance:

(i) The re-organization of the department with an increase in full-time staff.
(ii) A better system of referring from the screening department.
(iii) An increase in number of consultations for minor ailments.
(iv) An increasing awareness amongst the population of the facilities offered by the hospital.

The monthly figures show a slight increase in summer, a not excessive decline in autumn, a sharp increase in winter and finally an irregular decrease in spring. These findings are similar to those reported elsewhere, and they emphasize the relationship between the direct and indirect climatic factors. In Johannesburg the summer climate is characterized by short periods of extreme heat and heavy rainfall, and sometimes by periods of drought. These conditions inevitably lead to alterations in the patterns of the sweat function and evaporation, in sebaceous secretion and in skin pH. In turn clothing, food intake, use of cosmetics and moisturing agents, and the abundance of micro-organisms and parasites pathogenic to the skin are affected. The increase in winter months is difficult to interpret. We can only speculate on the possible effect of other systemic diseases, common in winter, on the immune balance of patients, the abuse of medicaments for their treatment, the decrease of hygienic care and the changes in diet and clothing.

From all these figures we have selected fourteen of the most common skin diseases, reporting them in detail. Each condition is represented as follows:

(i) Monthly, yearly and 3-year figures.
(ii) Percentage of attendance for each condition in relation to that for all consultations.

Acne

The average was 2·86%. It was more frequent between the ages of 11 and 30, with slight female preponderance and with a tiny increase in the spring and summer months.

Drug eruptions

The average was 1·22% which is lower than that found by the FDA in USA (5%). They were more frequent between the ages of 11 and 50, and equally distributed between both sexes. There was a slight increase in the figures for the spring and summer months and also in those for 1972. Phenolphthalein containing laxatives were the most frequently incriminated causes of these ailments, and were followed by analgesics and various herbs.

Bacterial infections

The average was 16·56%. This figure is much lower than that reported by Desai (1972) from India (40–60%), but his statistics included scabies and mycotic infections. We found them to be most common in childhood, although they were seen up to the age of 50 years. The causative agents were usually staphylococci and streptococci. Systemic complications were rare.

Condylomata acuminata

The average was 5·21%. Condylomata acuminata were seen in children but they were more frequent between the ages of 11 and 30. A slight preponderance in males with an increase in the spring and summer months, and an evident decline in 1972 were noted. In the females they showed a tendency to become hypertrophic. Relapse after radical treatment was rare.

Contact dermatitis

The average was 9·29%. This condition was seen in children, but it has shown a remarkable increase between the ages of 11 and 40 and in the last period of 2 years. It was more frequent in females and in the spring and summer months. Occupational dermatoses were rare and mainly observed in mechanics and in rubber workers. Contact dermatitis caused by modern detergents and bleaching materials is on the increase in domestic servants and housewives. Cosmetic reactions were responsible for eczema-like eruptions localized to the light-exposed areas, or to other sites. Primary irritant effects were commonly seen after the use of deodorants for axillae (in women) but a few cases were found amongst men. Perfumes were also contributory but the active ingredient cannot be presented. Photocontact dermatitis was seen in several patients; although the skin of our black African seems to be well protected against the effects of chronic exposure to sunlight, it is frequently affected by the use of photosensitizers incorporated into various medicaments, as well as into cosmetic preparations. Photopatch tests were carried out only occasionally, but the results were extremely disappointing. We believe that the wide range of chemicals involved, the cross-reaction between the medicaments used topically and systemically by our patients, and the difficulty in collecting the history may all account for our unsuccessful findings.

Wigs, worn by the majority of African women, were causative factors in producing erythematous–squamous eruptions confined to the border of the hair. This condition was probably related to the fabrics or to azo-dyes used in the manufacture of wigs.

Eczema

The average was 13·25%. Eczema affected children as well as adults and was most frequently seen between the ages of 21 and 60. It was more common in males and during spring and summer months.

Superficial mycotic infections

The average was 5·93%. These cutaneous infections were found predominantly in children and between the ages of 21 and 40, and equally distributed in both sexes. There was an increase in incidence during spring and summer months.

In order to characterize the dermatophytes causing tinea capitis in the African children we have conducted a survey in the schools and crèches of Soweto, the urban African township. The screening of 5085 pupils showed that 263 of them had scalp lesions compatible with the clinical diagnosis of tinea. Only 223 specimens were found positive on microscopy and culture. The analysis of our results shows that pupils of 2 – 6 years appear to be more susceptible and that *T. Violaceum* plays the major role. It is followed by *M. Audouinii*, whereas *M. Canis* is negligible (Dogliotti & Bisset, 1974).

Pellagra

The average was 1·98%. Pellagra was rare in childhood, and showed a high incidence between the ages of 20 and 50. It was more prevalent in males, increased during spring and summer months, and decreased in 1972. In the urban Africans, pellagra was related more to consumption of alcohol than to nutritional deficiency. The clinical manifestations were usually confined to the light-exposed areas of the body, and were mainly represented by scaling, hyperpigmentation, bullae and intertrigo. Associated mucosal lesions such as glossitis, gingivitis and cheilitis were common. Gastro-enteritis, polyneuritis and dementia were uncommon. In our figures we have included two cases of pellagra induced by competitive antagonists of nicotinic acid (isonicotinic acid).

Psoriasis

Psoriasis has been described as rare in West Africa, but more frequent in East Africa. In our S. African blacks the average incidence was 1·68% which agrees with that of Farber & Petersen (1968) from USA (1–2%). In our survey psoriasis was rare in children but more common in adults between the ages of 20 and 50. It was seen prevalently in males and showed an increase in spring and summer months. It declined in frequency in the last 2 years.

Scabies

The mean average was 1·85% but in 1971 this increased to 2·14%. The cyclic variation in incidence of this parasitic infestation is well established. Its incidence has increased markedly in many parts of the world in recent years. This consideration led us to take especial care in the diagnosis of this condition. Scabies occurred mainly in the first two decades of life. Both sexes were equally affected, and there was an increased incidence in the autumn and winter.

Primary syphilis

The average was 9·80%. There was a progressive increase in 1970 and it has shown a predilection for colder months. The vast majority of patients were male.

Secondary syphilis

The average was 9·43%, but there was a marked decline in the last 2 years of study. It was rare in children and reached a peak in the second decade. The large majority of cases occurred in women. There was a marked increase in colder months.

Summing up the figures for early syphilis it is evident that this infection accounts for an average of 19·23% of all attendances. It is obviously a very high figure when compared with those presented by other countries. Nevertheless, we believe that this is an accurate reflection of the true incidence because there is no social stigma attached to attending our clinic, and it is well known throughout the population that facilities granted are free, that follow-up is based on an educational programme, and that the secrecy of the patient's ailment is very strict. Therefore patients are not tempted to seek advice elsewhere. During the 3 years under study a trend was noticed in which the incidence of primary infection increased whilst that of secondary declined. This trend may be accounted for by a number of reasons: a greater awareness by patients of the social and physical implications of allowing their sickness to remain untreated; a greater familiarity with the medical facilities that are available; and the tendency for all more socially advanced communities to demand more assistance and at an earlier stage of their ailments.

Urticaria

The average was 2·13% which is similar to the figure reported by Helgren & Hersle (1964). It was common in children and it showed peaks in the second and fourth decades. Women were mainly affected. There was an increase in the summer and autumn months.

Gonorrhoea

The average was 3·49%. It was most commonly seen in the second and third decades and there was a marked preponderance in males. It was frequent in colder months, and in 1972 there was an abrupt increase.

CONCLUSION

We were aware that a survey of morbidity and distribution of skin diseases occurring in a circumscribed area would have led to a 'single' spot survey, that Marshall describes as being of limited value; but we were encouraged by the statement of Canizares 'that modern epidemiology means essentially the study of the ecology of diseases', meaning the circumstances under which diseases occur, where the diseases tend to flourish, and where they do not occur.

Although numerous and different methods of investigation have been suggested and developed, none appears to be entirely satisfactory. Clinical epidemiology deals primarily with limited groups, such as family or small communities, which may come under observation. This way of collecting data may lead to differences, as do other factors such as an insufficient classification, inaccurate registration of cases, inadequate follow-up, lack of continuity in make-up of the investigating team, notable variations in attendance and differences in the standard of living of the groups.

Taking into due consideration these causes of error, we have conducted our study over a relatively long period, in a homogeneous urban population of black Africans resident in temperate climate conditions, divided into various age and sex groups and attending a single institution. We do not pretend to have solved all the problems related to the incidence of skin diseases involving the black population of South Africa, but we believe we have followed some selective criteria able to minimize the errors.

To offer an acceptable explanation of all the findings reported in the Tables would be extremely ambitious, nevertheless we can shed light on certain data on clinical and epidemiological grounds. For example, we were able to detect some skin disorders described in previous papers as non-existent or very rare in the African Negro and notably in the S. African blacks. They are referred to in Table 2. Amongst them we have recorded 3 cases of lichen sclerosus et atrophicus, rarely seen previously in Africa (Scott & Lups, 1971, Dogliotti *et al.*, 1974), one case of leprochaunism (the seventh in the literature), one case of lupus erythematosus profundus (Dogliotti *et al.*, 1974), three cases of multiple xanthomatosis, and one case of generalized and extensive neurofibromatosis. In addition we can confirm that our black patients can present with exuberant and diffuse manifestations of lichen planus.

TABLE 2. Rare skin disorders in the black South Africans (1969–1972)

Diagnosis	No.	Age	M	F	Complications of	
Adenoma sebaceum	4	16–32	2	2	None	
Basal cell epithelioma	1	39	1	—	Albinism	
Bowen's disease	1	31	—	1	None	
Chancroid	1	35	—	1	None	
Dermatomyositis	1	36	—	1	None	
Epidermolysis bullosa	3	0–20	3	—	None	
Hydroadenoma eruptivum	3	13–45	2	1	None	
Larva migrans	10	0–15	5	5	None	
Leprochaunism (undescribed)	1	4 months		1	CHO metabolic derangement	
Lichen sclerosus and atrophicus	4	17–35	1	3	None	
Lupus erythematosus profundus	1	19	—	1	None	
Protothecosis	1	49	1	—	None	
Pseudopelade	2	36–38	2	—	None	
Sarcoidosis	6	21–60	—	6	None	
Scleroderma	8	14–39	2	6	None	
Xanthomatosis	3	39–65	2	1	Biliary	
					Cirrhosis	M
					Diabetes	M
					None	F

Its incidence, 0·78%, is far lower than that reported by Privat & Faye (1967) from Senegal (5%), and it was even lower than that reported by Femi Soyinka (1973) from Nigeria (1–15%); our urban black patients showed buccal mucosal lesions in 10% of cases. This figure, lower than that reported by Samman (1962) from London, leads us to raise objections to the alleged rarity amongst African Negroes.

Paying attention to the dynamic influence of the ecology on the incidence of skin diseases, it seems that the variations in attendance and frequency may be related mainly to climatic factors and customs affecting this population. The other factors seem to be less significant. On the other hand the standard of education, the life-style, the personality and behaviour of the inhabitants may play a role. These elements may be responsible for an individual's adaptation and response to the stimuli and finally may influence or produce alterations in distribution and morbidity.

In addition to the above mentioned causes of variability of incidence there are some other factors to which we attach a paramount importance:

The permissivity of a rapidly developing society, the expanding economy, the changes in moral standards which have also affected the communities of tribal extraction, the ever-increasing promiscuity and certain 'marriages of convenience' promulgated to counteract loneliness and frustration. These elements may be of greater influence than poverty and ignorance.

These considerations may, perhaps, give a clue to the evident discrepancies of morbidity and incidence of these diseases reported from various areas of this continent.

According to our experience some common errors, leading to inconsistent results, become negligible when the data are collected in a fully organized and equipped institution, and when the following details are taken into account:

(i) Accurate registration of facts observed in a population as large and as homogeneous as possible.
(ii) Appropriate use of classification by a constant team.

(iii) Adequate extension of the period of study to permit the various influencing factors to act sufficiently to demonstrate their effects.

Finally we feel it is important to collate data obtainable from individual localities, and perhaps, to present them at a symposium to be included at each international congress of dermatology. Only in this way can an overall impression of the problem of an entire continent be obtained.

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A method of liberating living cells from the dermal infiltrate

STUDIES ON SKIN RETICULOSES AND LICHEN PLANUS

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Accepted for publication 9 April 1975

SUMMARY

A new technique is described whereby viable infiltrating cells can be freed from skin biopsy specimens. The specimens are incubated with collagenase and then mechanically disaggregated. The liberated cells are still suitable for immunological and morphological study. Using this method, the nature of the dermal infiltrate in patients with skin reticuloses was compared with that in lichen planus. A predominance of T cells was found in mycosis fungoides, the Sézary syndrome, and lichen planus, and of B cells in non-Hodgkin lymphomas.

The nature of the lymphoid cells in the dermal infiltrate is of importance in the pathogenesis of skin diseases. Using known differences in cell membrane receptors, B cells can be identified in frozen tissue sections (Edelson *et al.*, 1973). However, B cells are more usually identified living in suspension. As yet, there is no comparable method of identifying T cells in tissue sections; they can only be identified living in suspension (Coombs *et al.*, 1970). Mechanical methods of tissue disaggregation have been applied by Edelson *et al.* (1974) and Tan *et al.* (1974) in their studies of the skin lesions of mycosis fungoides. These methods alone are inefficient unless the infiltrate is so dense that it virtually replaces the collagen fibres. Prior digestion of the tissue with trypsin would be effective, but this enzyme removes some cell surface antigenic determinants (Fakhri & Tan, 1975) and abolishes the ability of lymphocytes to form spontaneous rosettes with sheep red cells (Jondal, Holm & Wigzell, 1972).

We describe a technique whereby skin biopsy specimens are incubated with collagenase and then mechanically disaggregated to liberate viable cells—sufficient in number and still suitable for immunological and morphological study.

PATIENTS AND METHODS

Sixteen patients were studied (Table 1). They were selected from those attending Dr P.D.Samman's

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TABLE 1. Details of patients and nature of lymphoid cells in the skin infiltrate

Patient no.	Age	Sex	Diagnosis/stage	Histopathological diagnosis	Total no. of cells liberated $\times 10^6$	% T cells in infiltrate	% B cells in infiltrate
1	76	F	MF tumour	MF	4·2	69	< 5
2	45	M	MF tumour	†MF	4·5	66	< 5
3	62	M	MF tumour	MF	8·0	78	< 5
4	70	M	MF plaque	MF	4·0	86	None detected
5	73	F	MF premycosis	Eczema	0·15	70	None detected
6	67	F	Sézary syndrome	†Sézary syndrome	1·4	86	None detected
7	38	M	Sézary syndrome	†Sézary syndrome	0·46	68	None detected
8	54	M	Lymphoma	Lymphoma	1·7	33	51
9	58	F	Lymphosarcoma	Lymphosarcoma	16·0	58*	None detected*
10	74	M	Lymphoma	Lymphoma	1·2	83	None detected
11	62	F	Lymphoma	Lymphocytic lymphoma	17·5	39	60
12	61	F	LP	LP	0·6	67	None detected
13	65	M	LP	LP	0·13	70	< 5
14	54	F	LP	LP	0·32	78	None detected
15	41	F	LP	LP	0·22	84	None detected
16	64	M	LP	LP	0·55	82	Not done

* 38% of cells were large atypical mononuclear cells devoid of any detectable surface markers.
† Compatible with.
MF, Mycosis fungoides.
LP, Lichen planus.

reticulosis clinic at St John's Hospital for Diseases of the Skin, or from those attending dermatology clinics at Westminster Hospital. There were five patients with mycosis fungoides at the tumour or plaque stage (Samman, 1970), two with the Sézary syndrome confirmed by electron microscopy of circulating white cells (Zucker-Franklin, 1974a), four with lymphomas confirmed by skin histopathology and five patients with lichen planus. Control studies were undertaken on two similar sized pieces of normal skin removed during surgical operations.

Liberation of infiltrating cells

An approximately 1·5 cm elliptical skin biopsy was taken under local anaesthetic. One third was sent for histopathology. The remainder was chopped finely with a scalpel in 10 ml Eagle's minimal essential medium (MEM) containing 0·25 M Hepes buffer, 5 mg collagenase (Sigma, London), 0·1 ml of 0·5% calcium chloride, penicillin (100 units/ml), streptomycin (100 μg/ml) and Fungizone (0·25 μg/ml). It was then left at 4°C overnight. The next morning it was disaggregated with a magnetic stirrer for 1$\frac{1}{2}$ h at 37°C, and further disaggregated for 5 min by suction and expulsion through a standard 20 ml needle-less syringe. The resulting suspension was filtered through sterile gauze to remove tissue remnants and washed through with fresh MEM. The filtrate was centrifuged at 1500 rev/min for 10 min and washed with MEM containing 10% heat inactivated fetal calf serum (IFCS). The resulting cells were counted and viability was assessed by the trypan blue exclusion technique.

T cell enumeration

An aliquot of the cell suspension was mixed with washed sheep red cells (SRBC) (Wellcome Reagents Ltd.) suspended in IFCS, giving a final ratio of approximately 30–50 SRBC/skin cell. After incubation at 37°C for 15 min, the cells were centrifuged at 800 rev/min at 4°C for 10 min and then left at 4°C overnight before counting. The cells were then gently resuspended by shaking and examined by phase contrast microscopy. One hundred to 200 small to medium sized mononuclear cells were counted. Cells binding three or more SRBCs were considered positive. In some cases large mononuclear cells were also counted and recorded separately.

B cell enumeration

A second aliquot of the cell suspension was incubated at 4°C for 20 min with 0·1 ml polyvalent fluorescein conjugated goat anti-human Ig serum (anti-IgG, IgA, IgM, H and L chains, Behringwerke AG) diluted 1:8. The cells were then washed 3 times with phosphate buffered saline at 4°C and subsequently kept on ice until examined with a Leitz orthoplan microscope equipped with a mercury vapour HB 200 lamp and fluorescence vertical illuminator. Fifty to 100 cells were counted and, in addition, the whole field was scanned under ultraviolet light. Cells with discrete fluorescing spots scattered on their surface membrane were considered positive.

Cytology

Preparations of freshly liberated infiltrating cells and rosettes were made on microscope slides using a cytocentrifuge (Shandon, Elliott). They were fixed in methanol, stained with Wright's stain and examined by light microscopy with an oil immersion lens.

RESULTS

The number of mononuclear cells liberated from the skin biopsy specimens is given in Table 1. These counts record the number of lymphoid cells, but may also include other mononuclear cells such as monocytes, histiocytes and even epidermal cells which become rounded when freshly disaggregated. The viability of the cells was over 85% in every case and usually greater than 90%. The proportion of T and B cells found in the infiltrate is given in Table 1. Almost all the T cell rosettes contained over 15 SRBC (Fig. 1); they were easy to identify. B cells were more difficult to identify as their plasma

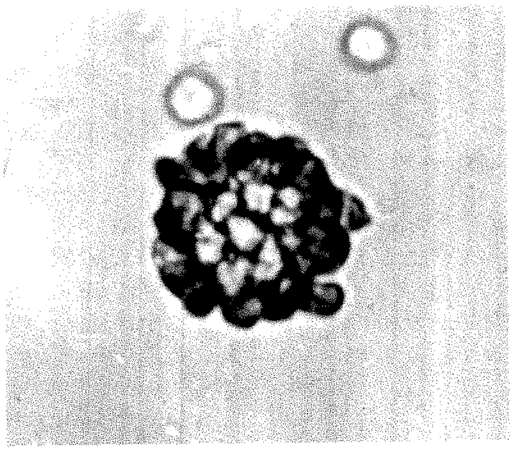

FIGURE 1. Wet preparation of a lymphocyte, freshly liberated from the cutaneous infiltrate, surrounded by a tightly packed rosette of sheep red cells (× 1000).

membrane fluorescence was short lived. Large mononuclear cells never formed rosettes and never had detectable surface immunoglobulin.

In all patients with MF, the Sézary syndrome and lichen planus, most of the infiltrating cells were T cells; few B cells were detected. However, in two patients with lymphoma at least half the infiltrating cells were obviously B cells; these cells had many brightly fluorescing spots scattered over the surface of their plasma membrane. A study of the morphology of 'rosetting' cells and other cells in the dermal infiltrate will be reported in full at a later date. Fig. 2 shows a 'rosetting' cell from the skin of a patient with the Sézary syndrome. The folded nature of the cell nucleus is evident.

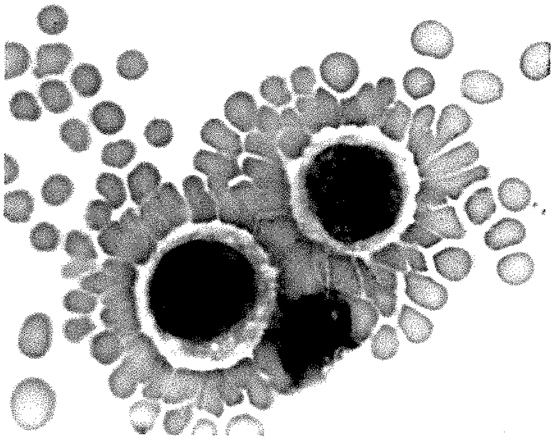

FIGURE 2. Stained cytocentrifuge preparation of an abnormal T lymphocyte, with a deeply folded nucleus, from the skin infiltrate of a patient with the Sézary syndrome (× 1000).

DISCUSSION

The number of cells freed from the skin depends upon the density of the infiltrate. The greatest yield was from patients with malignant lymphomas in whom the dermal collagen was all but replaced by the infiltrating cells; the smallest yield was from patients with lichen planus already treated with topical steroid creams. However, in all cases sufficient numbers of viable lymphocytes were available for their immunological status to be determined and for subsequent morphological study.

The proportion of the infiltrating lymphocytes that are T cells in the patients with MF and with the Sézary syndrome is likely to be greater than the figures given in Table 1. This is because all small to medium sized mononuclear cells were counted. The count would therefore include infiltrating monocytes and macrophages, which are often indistinguishable morphologically from lymphocytes (Hansen & Good, 1974; Zucker-Franklin, 1974b). Our results support the belief that mycosis fungoides and the Sézary syndrome are disorders of T cells, as the majority of infiltrating cells formed spontaneous rosettes with SRBC and only very few cells had detectable surface immunoglobulin. The T cells in the infiltrate could be reacting cells and not neoplastic cells. We have, however, already shown abnormal T cells in the skin infiltrate of patient 6 with the Sézary syndrome (Fig. 2), who was demonstrated at the December meeting of the Section of Dermatology at the Royal Society of Medicine and subsequently reported by Tan *et al.* (1975).

The majority of the infiltrating lymphoid cells in patients 8 and 11, both with lymphoma, had detectable surface bound immunoglobulin. In patient 9, many large mononuclear cells were present, showing indented nuclei and considerable quantities of pale eosinophilic material in the Golgi zone on Romanowsky staining. The appearance suggested a possible origin from the B cell series, but surface immunoglobulins were not detected. However, some B cells, although lacking detectable surface immunoglobulin, may still have other characteristic B cell markers, such as receptors for C_3 (Ross *et al.*, 1973), for which we did not test. Our findings support the belief that some non-Hodgkin lymphomas are disorders of B cells (Hansen & Good, 1974; Aisenberg & Bloch, 1972). Many T cells also were detected in these patients; they were probably cells reacting against the 'neoplastic' B cells.

Our preliminary findings in lichen planus indicate that the infiltrating cells are predominantly T cells. B cells would seem to play little or no part in the pathogenesis of the disease. We intend to undertake cytological and ultrastructural studies of these cells and compare them with those freed from the dermal infiltrate of the skin reticuloses.

Our results prompt us to speculate that the predominant reacting lymphocyte in the skin is the T cell. If this is confirmed in other dermatoses, the skin might well be an important extra-nodal site for the 'homing' of T cells (Goudie, Macfarlane & Lindsay, 1974), and so could be regarded as an organ of immunological surveillance.

ACKNOWLEDGMENTS

R.S-H.Tan was supported by the Governors' Discretionary Fund of the Westminster Group of Hospitals and N.A.Byrom by a grant from the Cancer Research Campaign.

We would like to thank Dr P.D.Samman and Dr P.W.M.Copeman for allowing us to study their patients, and to thank them and Professor J.R.Hobbs for their invaluable help and encouragement. We would also like to thank Mr A.R.Williams for taking and processing the photomicrographs.

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Thin layer chromatography and counter-current analysis in porphyrias

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Accepted for publication 11 November 1974

SUMMARY

The porphyrin metabolism of 100 patients with porphyria and 351 of their relatives has been studied. Thin layer chromatography of methyl esters of the urinary porphyrin was undertaken in sixty-six patients with different types of porphyria, and forty-five relatives, seventeen patients with hepatic cirrhosis, three patients with lead poisoning and twenty normal control subjects. This investigation was also made on the stools of thirty-six patients with porphyria, and ten of their relatives. Counter-current analysis of the bile of nine selected patients with porphyria was also undertaken.

The results provide some evidence that symptomatic hepatic porphyria may be familial. Thin layer chromatography was decisive in the characterization of a new type of porphyria described recently by the authors (hepato-erythrocytic porphyria). The counter-current examination of the bile showed the absence of the 'S 411' porphyrin in all the nine cases investigated.

The current classification of the porphyrias has been made by studies of the clinical, biochemical and aetiological features of each type of porphyria. Inevitably, some key questions remain unanswered. For example, we do not know whether all cases of symptomatic porphyria (SP) are produced exclusively by a toxic agent acting occasionally or repeatedly on normal hepatocytes. An alternative hypothesis is that the toxin acts on abnormal liver cells with structural or functional anomalies of genetic origin. Symptomatic porphyria usually becomes obvious during adult life, as a consequence it is sometimes called porphyria cutanea tarda (PCT). However, there are cases described with an early onset and with no definitive evidence of exposure to liver toxins. For these cases we will use the term 'infantile porphyria'. In these cases, a familial influence is presumable, although little evidence has so far been presented to support this contention (Mazza, Battistini & Prato, 1971). Some cases of SP with characteristic clinical symptoms show deviations from the accepted pattern of porphyrin excretion. In view of these observations investigation of possible 'carriers' of the porphyric genetic trait is mandatory.

In this paper we present data from patients examined over the last 5 years (1969–1974) in the Department of Dermatology of the University of Barcelona. The porphyrin metabolism of each patient and all his or her available relatives has been studied and is described. These results are compared with those from patients with liver cirrhosis and with lead poisoning.

The porphyrin excretion patterns have been established by use of thin layer chromatography (TLC) and counter-current (CC) fractionation. Extracts from urine and faeces have been examined, also from bile in selected cases.

Finally, we report a hitherto undescribed type of porphyria recently investigated in our department.

MATERIALS AND METHODS

This study was carried out on 100 porphyric patients and 351 of their relatives, on seventeen patients with liver cirrhosis, on three with lead poisoning and on twenty normal persons.

The 100 porphyric patients were diagnosed as shown in Table 1.

TABLE 1

	No. of cases
Symptomatic porphyria (SP)	83
Variegate porphyria (VP)	5
Infantile porphyria (IP)	3
Hereditary coproporphyria (HCP)	1
Erythropoietic protoporphyria (EPP)	6
Hepato-erythrocytic porphyria (HEP)	2
Total	100

Specimens of urine and faeces were either extracted immediately after collection or stored at −4°C. Blood was assayed fresh. Bile in the form of duodenal contents was obtained by duodenal catheter (Salem or Levy type). One hour before catheterization, metoclopramide was injected, the injection being repeated 10 min before the extraction. The catheter was localized in the duodenum by means of X-rays, and 10–20 ml of magnesium sulphate solution was injected through the catheter.

Quantitative estimations of blood, faecal and urinary porphyrins were done using the techniques described by Rimington (1961).

The urinary porphyrins were extracted and prepared for TLC by adjustment of an aliquot from a 24 h collection to pH 3·4 and absorption on to talc. The dried talc was treated with methanol and sulphuric acid (95:5, v/v.) for 20 h to convert the porphyrins to their methyl esters. The esters were washed with 2% ammonia aqueous solution to remove unesterified material, then taken down to dryness in a rotary film evaporator. TLC was carried out on silica gel plates (0·3 mm thick Merck). The single or 2-dimensional systems of Smith (1975) were used. Quantification was achieved by elution of the porphyrin bands with 3 ml of chloroform, centrifugation and spectrophotometric analysis.

The method used for the extraction and examination of the faecal samples was the homogenization technique of Elder & Path (1971), the porphyrin esters being chromatographed in the same way as the urine esters.

Specimens of bile were extracted and the free porphyrins fractioned on a Graig 120 tube microscale counter-current apparatus using the technique of French & Thonger (1966). The porphyrins of the fractions were separated and identified by their chromatographic, spectroscopic and chemical behaviour (Smith, Belcher & Mahler, 1971).

RESULTS

(1) *Screening and quantitative determination of porphyrins in relatives*

(A) *Relatives of patients with SP.* Investigations were carried out on the urine, blood and stools of 308 family members of our SP patients. None of them showed abnormal levels of porphyrins or positive screening. Two of the patients of our series were brothers.

(B) *Relatives of patients with VP.* The same investigations were effected on fifteen clinically healthy individuals, members of the families of five patients. In each of these five families there were one or more relatives of the propositus with an abnormal protoporphyrin level in the stool. All the abnormal levels were observed in females.

(C) *Relatives of patients with HCP.* Only two members of the single case studied were investigated. In both, the porphyrin levels were normal.

(D) *Relatives of patients with EPP.* The investigations were carried out on ten members of three families. Only one showed abnormal levels of blood porphyrins.

(E) *Relatives of patients with IP.* Investigation of eleven members of the families of our three patients showed four subjects from two families with abnormal level of porphyrins in the stools.

(F) *Relatives of patients with HEP.* Investigations of five members of two families showed none with abnormal levels of porphyrin.

(2) *Thin layer chromatography of porphyrin esters*

The results are shown below:*

(A) *In the urine.* A total of 151 cases were investigated.

SP	56
VP	3
IP	2
EPP	2
HEP	2
HCP	1
Total porphyric	66 cases
Relatives of porphyric patients	45
Hepatic cirrhosis	17
Lead poisoning	3
Normal control subject	20

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* The following abbreviations have been used: PP, UP and CP = proto-, uro-, and coproporphyrin; 7COOH, 6COOH, 5COOH and 3COOH = hepta-, hexa-, penta- and tricarboxyl porphyrins; isocopro = isocoproporphyrin; patterns A_1 and A_2 were described by Doss *et al.* (1971) and porphyrins P_1 and P_2 by Elder & Path (1971).

The results were as follows:

(a) *Distribution of the porphyrins in patients with SP*

(1) With predominating uroporphyrin:

UP > 7COOH > CP > 6COOH > 5COOH	16
UP > 7COOH > CP > P_1 (Isocopro) > 6COOH = 5COOH	3
UP > 7COOH > 5COOH > 6COOH > CP	5
UP > 7COOH > 6COOH > CP > 5COOH	2
UP > 7COOH	1
	27 cases

(2) With predominating heptacarboxyl porphyrin:

7COOH > UP > 6COOH = 5COOH > CP	8
7COOH > UP > 6COOH = 5COOH > P_1 (Isocopro) > CP	1
7COOH > UP > CP > 5COOH = 6COOH	11
7COOH > UP	1
	21 cases

(3) With predominating coproporphyrin:

CP > UP > 7COOH > 6COOH = 5COOH	4
CP > 7COOH > UP > 6COOH = 5COOH	3
CP > 5COOH > 7COOH > UP > 6COOH	1
	8 cases

(b) *Distribution of the porphyrins in patients with VP*

CP > UP > 5COOH > 6COOH > 7COOH	1 case
CP > > UP > 7COOH > 5COOH > 6COOH	1 case
CP > > > UP > 5COOH > 7COOH > 6COOH	1 case

(c) *Distribution of the porphyrins in patients with ECP*

CP > > > UP > 5COOH > 6COOH	1 case

(d) *Distribution of the porphyrins in patients with EPP*

CP > > > UP = 7COOH = 5COOH > 6COOH	1 case
UP > 7COOH > CP	1 case

(e) *Distribution of the porphyrins in patients with LP*

CP > 5COOH > UP > 6COOH > 7COOH	1 case
UP > 7COOH > CP > > 6COOH > 5COOH	1 case

(f) *Distribution of the porphyrins in patients with HEP*

5COOH > UP > 6COOH	1 case
5COOH > CP > UP > 7COOH > P_1 (Isocopro)	1 case

(g) *Distribution of the porphyrins in relatives of porphyric patients*

(1) Relatives of patients with SP

CP	10 cases
CP > UP	4 cases
CP > UP > 7COOH	7 cases
CP > UP > 7COOH > 6COOH = 5COOH	5 cases
UP = CP > 7COOH	3 cases
UP > 7COOH > CP	2 cases

(2) Relatives of patients with VP

CP	1 case
CP > UP > 7COOH > 5COOH > 6COOH	1 case
UP > CP > 7COOH > 5COOH > 6COOH	1 case

(3) Relatives of patients with IP

CP	1 case
CP > > UP	2 cases
CP > > UP > 7COOH	1 case
CP > > UP > 7COOH > 5COOH, 6COOH	1 case

(4) Relatives of patients with HEP

UP > 7COOH > > CP	1 case
UP > CP > > 7COOH	1 case
CP > > > 7COOH > UP > 5COOH	1 case
CP > > > UP > 7COOH > 5COOH, 6COOH	1 case

(5) Relatives of patient with HCP

CP > > UP > 7COOH	1 case
CP > > 7COOH > UP	1 case

(h) *Distribution of the porphyrins in patients with hepatic cirrhosis*

Patients with a porphyrin excretion level higher than 100 μg/24 h

CP	4 cases
CP > UP(A_1 Doss's type)	4 cases
CP > UP > 7COOH > 5COOH (A_2 Doss's type)	3 cases
Patients with a porphyrin excretion insufficient for chromatography	6 cases

(i) *Distribution of the porphyrins in patients with lead poisoning*

CP	3 cases

(j) *Distribution of the porphyrins in twenty normal subjects with normal porphyrin excretion level*

CP	3 cases
CP > > UP	7 cases
CP > > UP > 7COOH	6 cases
CP > > UP > 7COOH > 5COOH = 6COOH	4 cases

Observation of normal patterns on routine examination of urine in Cardiff since 1967 confirm the distribution of patterns from the Barcelona controls.

(B) *In the stool.* The same investigation was effected in twenty-nine cases of SP, three of VP, one of EPP, one of HCP, two of HEP and in ten relatives.

(a) *Distribution of the spots in SP patients*

PP > CP > P_1 (Isocopro) > 5COOH > 6COOH > 7COOH > P_2	2 cases
PP > CP > P_1 (Isocopro) > 5COOH > 6COOH > 7COOH > UP > P_2	5 cases
CP > P_1 (Isocopro) > 5COOH > 6COOH > 7COOH > P_2	3 cases
CP > P_1 (Isocopro) > 5COOH > 6COOH > 7COOH > UP > P_2	2 cases
CP > 5COOH > P_1 (Isocopro) > 6COOH > 7COOH > UP > P_2	7 cases
P_1 (Isocopro) > CP > 5COOH > 6COOH > 7COOH > P_2	9 cases
CP > UP > 7COOH > 6COOH	1 case

(b) *Distribution of the spots in VP patients*

PP > CP > 3COOH > UP > 5COOH > 7COOH	1 case
PP > CP > 3COOH > UP	2 cases

(c) *Distribution of the spots in EPP patients*

PP > > CP	1 case

(d) *Distribution of the spots in HCP patients*

CP > PP > 5COOH > 6COOH > 7COOH > UP	1 case

(e) *Distribution of the spots in HEP patients*

P_1(Isocopro) > CP > 5COOH > 6COOH > P_2 > UP > 7COOH > PP	1 case
PP > P_1 (Isocopro) > CP > 7COOH > UP > 5COOH > 6COOH	1 case

(f) *Distribution of the spots in relatives of SP patients*

PP > CP	5 cases
CP > PP	1 case
PP > 7COOH > UP > CP	1 case
UP > 7COOH > CP > PP	1 case

(g) *Distribution of the spots in relatives of HCP*

CP > > PP > 3COOH	2 cases

(h) *Distribution of the spots in normal people*

A 'normal' pattern of porphyrin excretion was not found in non-porphyrics. In over eighty individuals studied in Cardiff and Barcelona a wide variety of patterns was seen.

PP was not often the predominant spot but in some extracts it was not detectable. CP was always present and was occasionally the main spot. UP, 7COOH, 6COOH and 5COOH porphyrins were sometimes present in small quantities. Mesoporphyrin, pemptoporphyrin and unidentified porphyrins were also present in variable amounts in some specimens, sometimes in excess of the PP fraction. (Mesoporphyrin is a porphyrin derived from Protoporphyrin IX (Fisher & Orth, 1937). Pemptoporphyrin is a recently isolated faecal metabolite (French *et al.*, 1964; Sano *et al.*, 1965).

(3) *Counter-current pattern of porphyrins extracted from bile*
A total number of nine cases were investigated as follows:

SP	7 cases
VP	1 case
EPP	1 case

According to Smith *et al.* (1971) normal human bile can be resolved into five porphyrin fractions on the counter-current apparatus (Fig. 1).

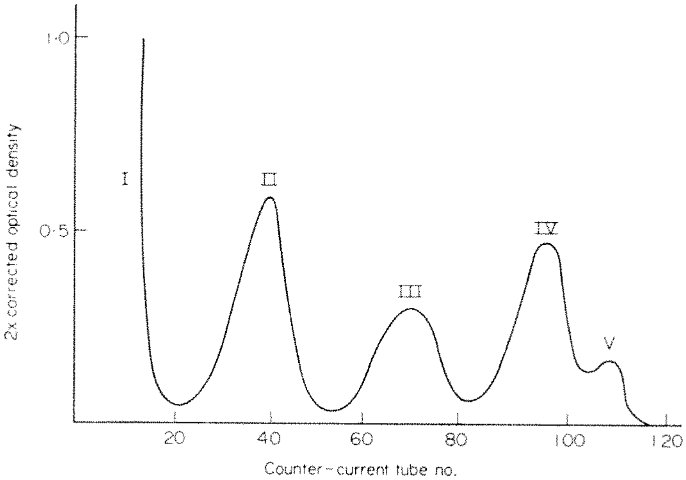

FIGURE 1. A typical counter-current fractionation of normal human bile.

Fraction 1 (peak I) was a mixture of CP I and III, the III isomer usually predominating.

Fraction 2 (peak II) consisted of: (a) a 2COOH porphyrin with an aetioporphyrin-type spectrum and a Soret band at 405 nm in 5% HCl (on reduction by catalytic hydrogenation this shortened to 402 nm); and (b) two 3COOH porphyrins both with Soret bands at 404·5 nm in 5% HCl reducible to 402 nm, and with aetioporphyrin-type spectra. The upper component on chromatograms was always the major 3COOH porphyrin. The 3COOH fraction was approximately 2 to 3 times the 2COOH fraction.

Fraction 3 (peak III) consisted of the 'S411' porphyrin with its characteristic Soret band at 411 nm, rhodoporphyrin-type spectrum, and ready convertibility by catalytic dehydrogenation to CP III.

Fraction 4 (peak IV) was PP.

Fraction 5 (peak V) showed chiefly degradation products of PP.

In the first case of SP the counter-current fraction pattern could be resolved as follows (Fig. 2):

Fraction 1 (peak I) was a mixture of 2COOH, 3COOH and 4COOH porphyrins with Soret bands at 405 nm in 5% HCl.

Fraction 2 (peak II) consisted of a 2COOH porphyrin with Soret band at 406 nm in 5% HCl.

Fraction 3 showed only small amounts of 2COOH porphyrin with Soret band at 406 nm in 5% HCl, 3COOH porphyrin with Soret band at 407 nm in 5% HCl and 4COOH porphyrin with Soret band at 405 nm in 5% HCl. 'S411' porphyrin was absent.

Fraction 4 (peak IV) contained 2COOH porphyrin with a Soret band at 405·5 nm in 5% HCl.

The counter-current fraction patterns of SP cases nos. 2–7 showed similar fractionation to case no. 1.

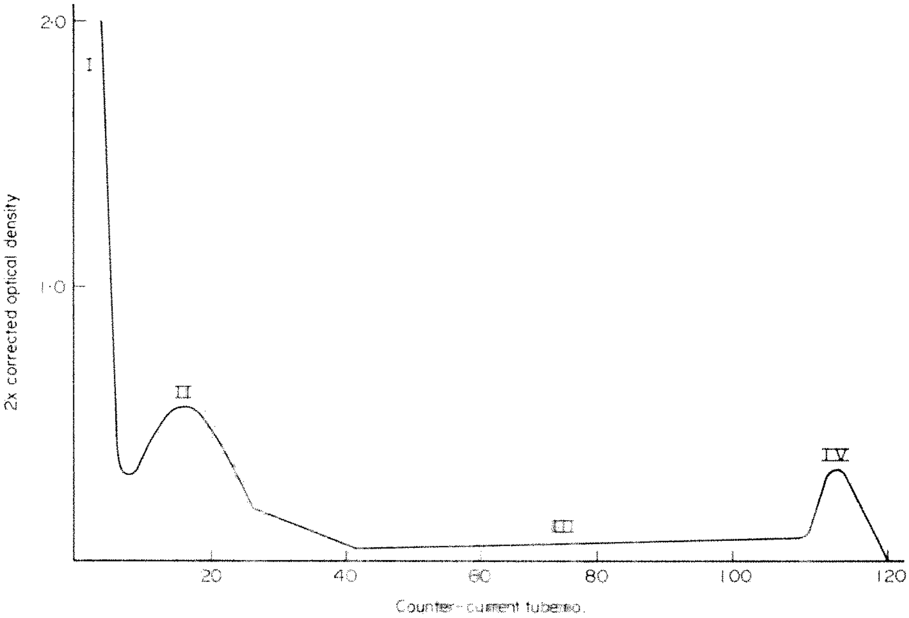

FIGURE 2. Counter-current fractionation of bile from a patient with SP (case no. 1).

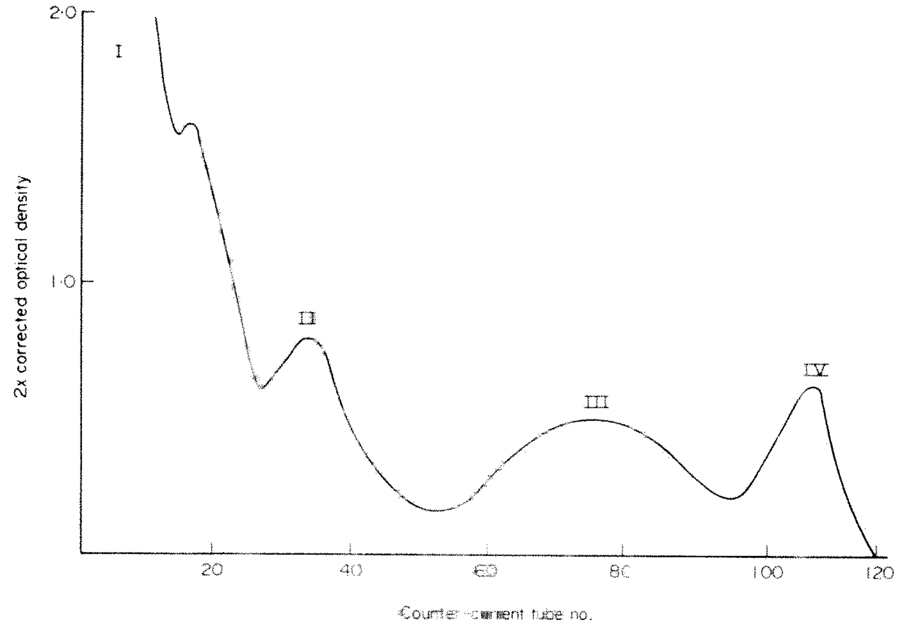

FIGURE 3. Counter-current fractionation from a patient with VP.

In the case of VP the pattern was as follows (Fig. 3):

Fraction 1 (peak I) consisted of: (a) a 2COOH porphyrin with a Soret band at 404 nm in 5% HCl; (b) a 3COOH porphyrin with a Soret band at 404 nm in 5% HCl and (c) a 4COOH porphyrin with a Soret band at 402 nm in 5% HCl.

Fraction 2 (peak II) consisted of a 3COOH porphyrin with Soret band at 404·5 nm in 5% HCl and a 4COOH porphyrin with Soret band at 403 nm in 5% HCl.

Fraction 3 (peak III) contained a 2COOH porphyrin with Soret band at 406 nm in 5% HCl and a 4COOH porphyrin with Soret band at 405 nm in 5% HCl. 'S411' porphyrin also was absent.

Fraction 4 (peak IV) contained a 2COOH porphyrin with Soret band at 406 nm in 5% HCl and a 4COOH porphyrin with Soret band at 408 nm in 5% HCl.

As in the three cases of VP studied by Smith *et al.* (1971) the 'S411' porphyrin was absent. Fig. 4 shows the counter-current fractionation pattern from one of these cases. Fraction 3 (peak III) contained only a 2COOH porphyrin with Soret band at 406 nm in 5% HCl.

In the case of EPP the counter-current fractionation pattern was as follows (Fig. 5):

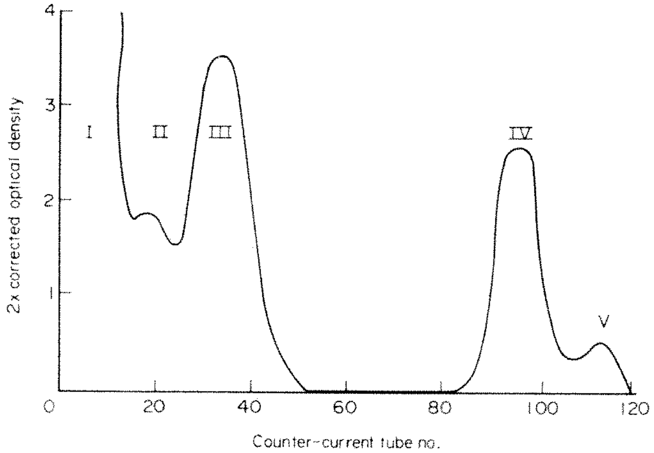

FIGURE 4. Counter-current fractionation pattern from a patient with VP (Smith *et al.*, 1971). Reproduced with kind permission of the South African Medical Journal.

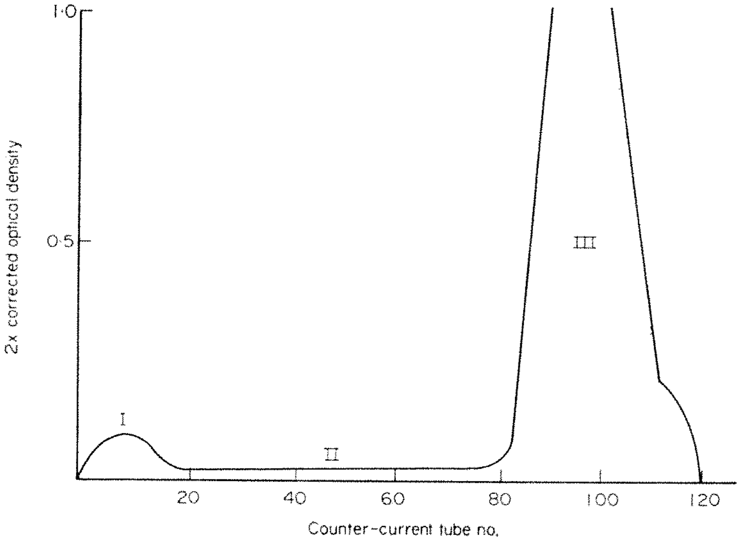

FIGURE 5. Counter-current fractionation pattern of bile from a patient with EPP.

Fraction 1 (peak I) consisted of a 4COOH porphyrin with Soret band at 403 nm in 5% HCl.

Fraction 2 (peak II was absent) showed only traces of 2COOH and 4COOH porphyrins with Soret bands at 407 nm and 403 nm in 5% HCl.

Fraction 3 (peak III) consisted of a 2COOH porphyrin with Soret band at 408 nm in 5% HCl.

The protoporphyrin was the main constituent of the tubes. Only small amounts of coproporphyrin were found (CP I 85% and CP III 15%). 'S411' porphyrin was absent.

DISCUSSION

A preliminary study by TLC of the urine and the faeces of the porphyric patients and their relatives and by the counter-current examination of the bile porphyrins was presented at the VII Spanish–Portuguese Congress of Dermatology, Funchal, October 1973. Here we present the same study with more data.

The value of the methods used in extending the knowledge of the pathogenic factors of the porphyrias is considerable. From the data produced in the present article it is possible to arrive at the following conclusions:

(a) The simple screening or the quantitative determination of porphyrins by solvent extraction and precursors in relatives of patients with SP *fails* to demonstrate a genetic transmission of this disease in the great majority of cases. Only two cases of our series were members of the same family (brothers) both showing clinical symptoms. On the contrary, in EPP, VP, infantile porphyria and HCP the routine determination of porphyrins by solvent extraction can be decisive.

(b) TLC of urinary porphyrins from our patients with SP showed the existence of two main excretion patterns in which UP and 7COOH porphyrins predominated. The first with UP greater than 7COOH porphyrin, and the second with 7COOH porphyrin greater than UP. In each case smaller and variable amounts of 4, 5 and 6COOH porphyrins were associated. Only eight cases of our series showed a predominant CP pattern. Thus, our findings agree with those of Sweeney (1963), Herbert (1966), Chu & Chu (1967), Simon, Dobozy & Berko (1970), Dowdle *et al.* (1970) and Doss *et al.* (1971). According to Doss *et al.* (1971) the raised heptacarboxyl porphyrin is pathognomonic of SP.

Interesting was the finding that in five of our thirty-one relatives of SP patients the excretion pattern of the porphyrins was UP predominating, although total porphyrins were found in normal levels; this pattern was different to that usually considered normal—CP predominating—the latter pattern being observed in our twenty normal control subjects. Thus there is some evidence that SP may be familial. However the number of cases studied and our control groups were too small to arrive at a firm conclusion.

(c) The urinary chromatography of our VP patients and their relatives also showed a predominant CP pattern. Only one member of the family of a VP showed a predominant UP pattern. One case of IP showed predominant CP pattern, and another UP pattern. Thus some cases of IP could be considered as true SP of early onset, but others could be familial.

(d) TLC was decisive in the characterization of the 'hepatoerythrocytic porphyria' a new type of porphyria recently described by us (Piñol Aguadé *et al.*, 1975). Till now four cases of this peculiar clinical picture have been reported (Piñol Aguadé *et al.*, 1969, 1974; Schneider & Simon 1974).

The main features are the early onset of symptoms, i.e. weeks or months after birth, the excretion of dark-coloured urine from birth, the photosensitivity with a tendency to mutiliation and scarring of the uncovered part of the skin, and marked hypertrichosis. There is evidence of liver disorder (the biopsy showed liver cirrhosis) but the spleen is not enlarged. From the biochemical point of view the disease is characterized by a raised red cell protoporphyrin, with occasional fluorescence of red blood cells and erythroblasts, and abnormal urinary porphyrins excretion with large amounts of 5COOH porphyrins. The pattern of porphyrin excretion in the stool is similar to that of SP.

This type of porphyria is different from that described by Hofstad, Seip & Eriksen (1973) and Eriksen & Eriksen (1974), in that they showed uroporphyrin and 7COOH to be the chief porphyrins in the urine. Our results showed a 5COOH porphyrin to be predominant. Also their coproporphyrin was 80% CPI, whereas our results showed 75% CPIII (Piñol Aguadé *et al.*, 1974).

(e) TLC of the faecal samples in SP confirms the findings of Elder & Path (1971) of the almost constant presence of the P_1 (isocoproporphyrin) and P_2; their position in the chromatogram is shown in Fig. 6.

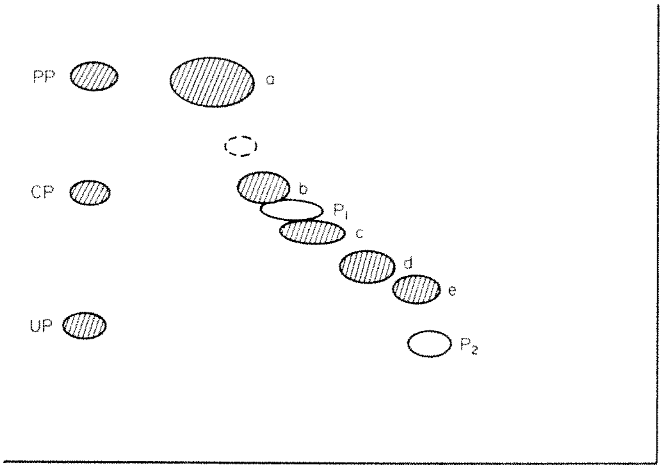

FIGURE 6. 2-dimensional thin layer chromatogram of porphyrin methyl esters from faeces in a patient with SP: P_1 = isocoproporphyrin; P_2 = Porphyrin P_2; a, b, c, d, e = Porphyrins with 2, 4, 5, 6 and 7 COOMe groups respectively. PP, CP, UP, controls. (From Elder, 1971.) Dashed-line encircled spots represent tricarboxyl porphyrin. Non-hatched encircled spots represent the abnormal porphyrins P_1 and P_2. Reproduced with kind permission from the South African Medical Journal.

It can be suspected that there is an interrelationship between these porphyrins and the isocoproporphyrin observed in the urine by us in some cases of SP and HEP. As occurred in the cases reported by Grosser & Eales (1973) in VP the 3COOH porphyrin was identified, but in SP was not detectable. The patterns of our VP also confirm the marked increase in 2COOH porphyrins and the lesser but nevertheless considerable increase of 4COOH porphyrins. In the examined case of infantile porphyria and in both cases of hepatoerythrocytic porphyria the excretion pattern was very similar to that of SP. In EPP, the 2COOH porphyrin constituted the main spot. In HCP the examination showed the presence of multiple porphyrins but the pattern differed from SP.

The TLC of the faecal samples of the relatives of SP gave no patterns similar to those indicated in the patients. In no case was isocoproporphyrin or P_2 found. Thus, this method seems of no use in detecting the possible 'carriers' of the disease.

With reference to the results of the current study of porphyrins extracted from bile, the most interesting finding was the absence of S411 porphyrins in the bile of SP patients and in the case of EPP investigated.

According to Smith *et al.* (1971), S411 porphyrin is a constant component of normal human bile. These authors found this porphyrin absent in VP, but our results showed that it is also absent in other types of porphyria. The role of this porphyrin in normal bile and its absence in porphyrias leave its significance open to conjecture.

It is also interesting to note that the biliary excretion of porphyrins in our case of EPP showed PP to be associated with only a trace of CP. In VP, where PP is being formed in excessive amounts in the liver, CP, 3COOH and 2COOH porphyrins are also found in excess. The CP found in VP is a mixture of I and III isomers, the III isomer predominating. This suggests that the biliary source of PP in EPP may be predominantly non-hepatic. This finding does not support the results of the investigations of Scholnick, Marver & Schmid (1971) on the two sources of protoporphyrin (erythroid cells and liver), but more extensive studies are needed.

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The use of thin layer chromatography in the separation of free porphyrins and porphyrin methyl esters

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Accepted for publication 28 February 1975

SUMMARY

Thin layer chromatographic techniques for the separation and subsequent quantification of free porphyrins, coproporphyrin isomers and the methyl esters of porphyrins are described including a 2-dimensional system. The relevant RFs for a wide range of porphyrins are given. These methods have been in use in Cardiff and Barcelona for a number of years and were used in the survey of cases described by Piñol *et al.* (1975) which resulted in the discovery of the new porphyria, hepato-erythrocytic porphyria.

The thin layer chromatography (TLC) techniques to be described were used in the survey of porphyrin excretion patterns from various porphyrics in Barcelona during the period 1971–1974 (Piñol *et al.*, 1975). They have also been widely used routinely and for research purposes in Cardiff for several years. The technique used for free porphyrins is based on the lutidine system described by Eriksen (1958) who used paper chromatography. Two methods of separation of porphyrin methyl esters are described.

Method (A) is similar to that described by Doss (1967) except that it is based on toluene in place of benzene.

Method (B) is derived from the system used by Chu, Green & Chu (1951) for paper chromatography of the porphyrin esters.

Methods (A) & (B) may be used sequentially in a 2-dimensional system which further improves separation.

The method described for the separation of coproporphyrin isomers uses the lutidine system of Jensen (1963) applied to cellulose coated TLC plates in place of silica gel coated plates.

Extraction and quantification of the separated porphyrins and porphyrin esters are also described.

MATERIALS AND METHODS

The porphyrins used as standards were taken from the laboratory stock with the exception of bis-hydroxy deuteroporphyrin, kindly supplied by Professor Sano, Kyoto University, and isocoproporphyrin kindly supplied by Dr G.Elder, Welsh National School of Medicine, Cardiff.

Three types of silica gel plates were used: (a) Merck (Kieselgel 60), (b) plates prepared on a Shandon plate spreader, (c) plates prepared on a Desaga spreader.

The laboratory-prepared plates utilized silica gel layers of 0·2 mm thickness. All plates were 20 × 20 cm. The tanks used were of dimensions 30 × 9 × 22 cm.

Separation of free porphyrins using silica gel plates

A well shaken solution of 50 ml distilled water in 100 ml 2:6 lutidine was poured into the tank which contained two small beakers of 30% v/v 0·880 ammonia solution in water. The tank was lined with two pieces of thick filter paper about 60 × 100 cm soaked in the 30% ammonia solution. The tank was put into a larger plastic tank containing water at about 40° C until the temperature of the chromatographic tank reached 22–24° C. It was then ready for use. The free porphyrins to be chromatographed were dissolved in a few drops of 2N NH_4OH and applied to the plate by micropipette. The time taken for a 10 cm run was about 2 h.

Separation of porphyrin methyl esters using silica gel plates

The methyl esters of the porphyrins were prepared by dissolving the free porphyrins in 5% conc. H_2SO_4 in methanol and allowing them to stand in the dark at room temperature for 24 h. An equal volume of distilled water was added to the esterification mixture and the porphyrin esters extracted with chloroform. The chloroform phase was separated and washed successively with 2% ammonia solution (v/v, to remove unesterified material) and distilled water. The resultant solution was evaporated to dryness on a rotary film evaporator.

System (A)

A well mixed solution containing 80 ml of toluene, 15 ml of ethyl acetate and 3 ml of ethanol was poured into the tank and allowed to equilibrate for 10 min.

System (B)

A well mixed solution of 60 ml of chloroform, 20 ml of redistilled kerosene and 4 ml of ethanol was poured into the tank and allowed to equilibrate for 30 min.

The porphyrin esters to be applied to the TLC plates in systems (A) & (B) were dissolved in small quantities of $CH\ Cl_3$ and applied by micropipette. The tanks should be freshly loaded with solvent each day. A 2-dimensional separation could be achieved by removing the developed plate from System (A), drying in hot air, and placing in the System (B) tank at 90° to the axis of the first run. The running time for both Systems (A) & (B) was 30–40 min each.

Separation of coproporphyrin isomers using cellulose plates

The tank was loaded with a well mixed solution of 30 ml of distilled water in 80 ml of 2:6 lutidine. Two small beakers containing SG 0·880 ammonia solution were placed in the bottom of the tank. The tank was allowed to equilibrate overnight and was then ready for use. The coproporphyrin mixture was dissolved in a small volume of 2N NH_4OH and applied to the plate. An excellent separation of the isomers was achieved in 4–6 h.

Quantification

The porphyrin bands were detected by their red fluorescence under u.v. light and identified by comparison with concurrently run standards. Each band was scraped off the plate and put into a stoppered centrifuge tube. The free porphyrins were eluted by first shaking with 3 ml of 20% acetic acid in ether, followed by a shaking with 1 ml of 5% HCl. After centrifuging, the fluorescent aqueous layer was transferred to a 1 cm path width microcell and read in the spectrophotometer. The porphyrin methyl esters were eluted in a similar way with 2 ml chloroform. Usually, one extraction was sufficient, but the elution procedure was repeated with heavily loaded bands using 2 ml of 50% $CH\ Cl_3$ in ethanol.

Relevant Soret band wavelengths, extinction coefficients and corrections for background absorption were described by Rimington (1958) and by Falk (1964). An assessment of the percentage of each porphyrin in a mixture, sufficiently accurate for routine purposes, may be made by a simple comparison of the Soret band absorptions.

All the porphyrins listed in Tables 1, 2 and 3 have been reported in human tissues or excreta with the exception of the hydroxylated porphyrins (2) and (3), and coproporphyrins II and IV.

The following RF values were recorded using systems (A) & (B) (Table 1) (Merck plates).

TABLE 1. RF values recorded using systems (A) & (B) (Merck plates)

Porphyrin Methyl ester		System (A) (155 mm run)	System (B) (132 mm run)
Haematoporphyrin	(1)	0·13	0·18
Bis-hydroxy deuteroporphyrin	(2)	0·14	0·22
Hydroxyethyl, ethyl deuteroporphyrin	(3)	0·28	0·39
Uroporphyrin	(4)	0·21	0·43
Heptacarboxyl porphyrin	(5)	0·25	0·47
Hexa carboxyl porphyrin	(6)	0·29	0·51
Penta carboxyl porphyrin	(7)	0·35	0·55
Isocoproporphyrin	(8)	0·39	0·57
Coproporphyrin I	(9)	0·45	0·58
Coproporphyrin III	(10)	0·46	0·58
Harderoporphyrin	(11)	0·51	0·62
Isoharderoporphyrin	(12)	0·52	0·62
Deuteroporphyrin	(13)	0·59	0·67
Protoporphyrin	(14)	0·62	0·68

RESULTS

The separation of the porphyrin methyl esters from an extract from the urine of a patient with hepato-erythrocytic porphyria (Piñol *et al.*, 1975) is shown in Fig. 1 and shows a typical separation using the 2-dimensional system.

Practical notes

The commercial Merck silica gel plates give sharper separations and allow the application of smaller spots or bands to the plates. Silica gel plates made in the laboratory using the Shandon or Desaga processes give softer layered plates from which porphyrins may be extracted more easily than from Merck plates.

The fluorescence of porphyrin esters after single runs in system B is more intense than after the system A development. It may therefore be preferred if photography is anticipated.

TABLE 2. RF values for various free porphyrins using the lutidine system with silica gel plates

Free porphyrin	RF value
Uroporphyrin	0·0
Heptacarboxyl porphyrin Hexacarboxyl porphyrin	0·05–0·15
Pentacarboxyl porphyrin	0·20
Coproporphyrin I	0·25
Coproporphyrin III	0·27
Harderoporphyrin	0·32
Isoharderoporphyrin	0·38
Protoporphyrin Deuteroporphyrin	0·47

System (A) is similar to that of Doss (1967) except that it gives a greater separation of hydroxylated porphyrin esters. These porphyrins may be further identified by the acetylation procedure of Barrett (1959), acetylation of the hydroxyl group(s) causing an increase in the RF value of the porphyrin. This system also largely avoids the health hazard of the benzene system of Doss, toluene being nearly ten times less toxic (Patty, 1963).

A clear unequivocal separation of the coproporphyrin isomers is best achieved by first separating coproporphyrin from other porphyrins in an extract. This may be done by using the first described system (for free porphyrins) preparatively. The extracted coproporphyrins may then be separated using the isomer system.

The use of the lutidine system for free porphyrins may be readily applied to extracts of porphyrin mixtures prior to methylation. It does not separate 5, 6, 7 and 8 carboxyl porphyrins as well as the

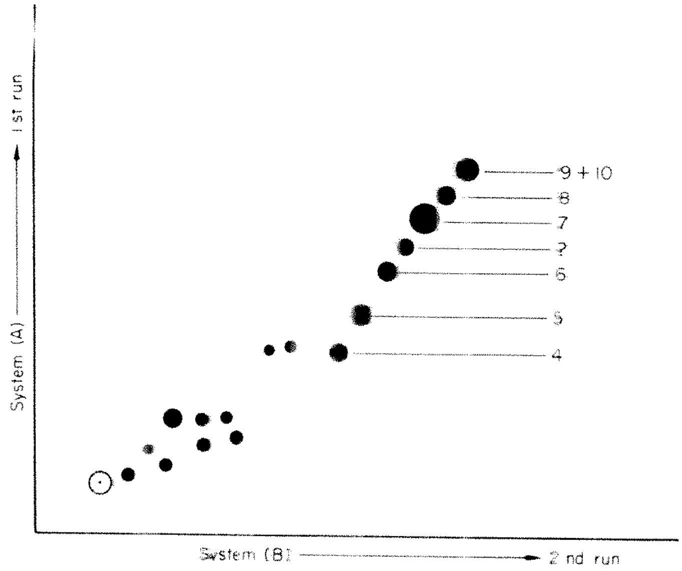

FIGURE 1. 2-dimensional separation of porphyrin esters from the urine extract from a patient with hepato-erythrocytic porphyria. (See Table 1 for numerical key.)

TABLE 3. Typical RF values for the separation of the coproporphyrin isomers after a 4 h 30 min run on Merck cellulose plates (0·10 mm thickness) using the lutidine system described for the separation of isomers

Porphyrin	RF value (4½ h run)
Coproporphyrin I	0·63
Coproporphyrins III and IV	0·50
Coproporphyrin II	0·43

ester systems, but allows separation of protoporphyrin and harderoporphyrins before methylation, a process which causes some losses of these porphyrins.

CONCLUSION

In order to arrive at the correct diagnosis of a porphyric, it is necessary to study the patient clinically and biochemically. A separation, identification and quantification of the porphyrins from urine, faeces and blood may be required, together with a coproporphyrin isomer analysis. In some cases it may be necessary to establish the isomer distribution of the hepta-, hexa- and penta-carboxyl porphyrins. This may be achieved by decarboxylation of the acetic acid side chains of these porphyrins (Falk, 1964) and subsequent coproporphyrin isomer analysis. The TLC systems described enable the relevant porphyrin separations to be carried out.

The use of these systems resulted in the discovery of a new type of porphyria, hepato-erythrocytic porphyrin (Piñol *et al.*, 1975).

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Further studies of scratching during sleep

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Accepted for publication 9 December 1974

SUMMARY

Fifteen patients with a variety of itching skin diseases (atopic eczema, dermatitis herpetiformis, lichen planus, urticaria and psoriasis) have been studied in the sleep laboratory. Recordings were made of all-night electroencephalogram, electro-oculogram, submental electromyogram, and muscle potentials from both forearms.

Bouts of scratching during orthodox (NREM) sleep occurred more frequently in stages 1 and 2 than in stages 3 and 4. The frequency in paradoxical (REM) sleep was close to that in stage 2 sleep. This pattern was similar for all the diseases studied and seems to be related to the physiology of the sleep stages rather than to the skin diseases themselves. The mean duration of the bouts of scratching was not related to the sleep stage in which they started.

We have previously reported (Savin, Paterson & Oswald, 1973) that patients with atopic eczema scratch during all stages of sleep, and that the incidence of scratch bouts varies in the different stages. We now report our findings on a larger group of patients with a variety of skin diseases.

Patients

Three groups of patients have been studied:

Group I. Five patients with atopic eczema: four for 1 night and one for 2 nights. Four of these patients formed the basis of our previous report.

Group II. Five patients with dermatitis herpetiformis: four for 2 nights and one for 1 night. The diagnosis in all cases had been confirmed by skin biopsy and response to dapsone.

Group III. A miscellaneous group of five patients for 1 night each. Three patients had lichen planus, one had urticaria, and one an itchy psoriasis.

METHODS

Patients were studied in the sleep laboratory as before (Savin, Paterson & Oswald, 1973). They slept in conditions of low illumination at a constant room temperature (18 °C) and remote from all recordings. The early patients in the series were observed on closed-circuit television allowing all movements, including scratching, to be noted by hand on the moving paper record. Our experience, gained in this way, allowed us to dispense with television after it became clear that bouts of scratching could be identified by their rhythmical quality using the recordings on the paper trace from the forearm leads. Most of the body movements during the night were associated with scratching.

Recordings were made of all-night electroencephalogram (EEG), electro-oculogram, submental electromyogram and muscle potentials from both forearms. Records were analysed in 20 s periods in terms of wakefulness and the usual stages of sleep: that is orthodox (non-rapid eye movement or NREM) sleep, with its stages, 1,2,3 and 4, and paradoxical (rapid eye movement or REM) sleep (Rechtschaffen & Kales, 1968).

The records were examined for:

(a) The duration of each bout of scratching.
(b) The stage of sleep in which the bout started.

Our method did not allow us to gain information about the amplitude of the scratching movements nor the frequency of the individual scratch strokes.

In addition we determined:

(c) The time spent in each sleep stage during the night.
(d) The number of bouts of scratching starting during each stage of sleep.
(e) The frequency with which bouts of scratching started during each stage.
(f) The duration of the bouts of scratching while asleep and while awake.

Several tests were used in the statistical examination of the data:

(1) Analysis of variance, a technique that allows a simultaneous comparison of more than 2 variables, was used to compare the duration of scratch bouts in the different stages of sleep.

(2) Correlated *t*-tests for paired observations in order to compare the duration of scratch bouts for each subject when in different sleep stages.

(3) Kendall's rank correlation test was used to investigate possible correlations between the durations, or between durations and frequencies, of scratching bouts in different stages of sleep. This test only takes into account the ordering and not the absolute values of the results.

RESULTS

Frequency of bouts starting in each stage of sleep

The results for the three groups of patients are shown in Fig. 1.

Scratching was most frequent in stage 1 and this was true of all three groups. The frequency decreased through stages 2,3 and 4. As we found before, REM sleep was in an intermediate position (Table 1). The difference between stages 1 and 2 was significant ($t=4{\cdot}06$, d.f. $=14$, $P<0{\cdot}01$). There was also a significant difference between stage 2 and stages 3 and 4 combined ($t=2{\cdot}64$, d.f. $=14$, $P<0{\cdot}02$). There was no significant difference between stage 2 and REM sleep, between stages 3 and 4 and REM sleep, or between wakefulness and stage 1.

Duration of bouts of scratching starting in each stage

The details of the three groups of patients are shown in Table 2.

There was no significant difference in the length of bouts starting in the different stages of sleep, when one-way analysis of variance was undertaken (d.f. $=3{\cdot}42$; $f=0{\cdot}112$; n.s.). Although intersubject

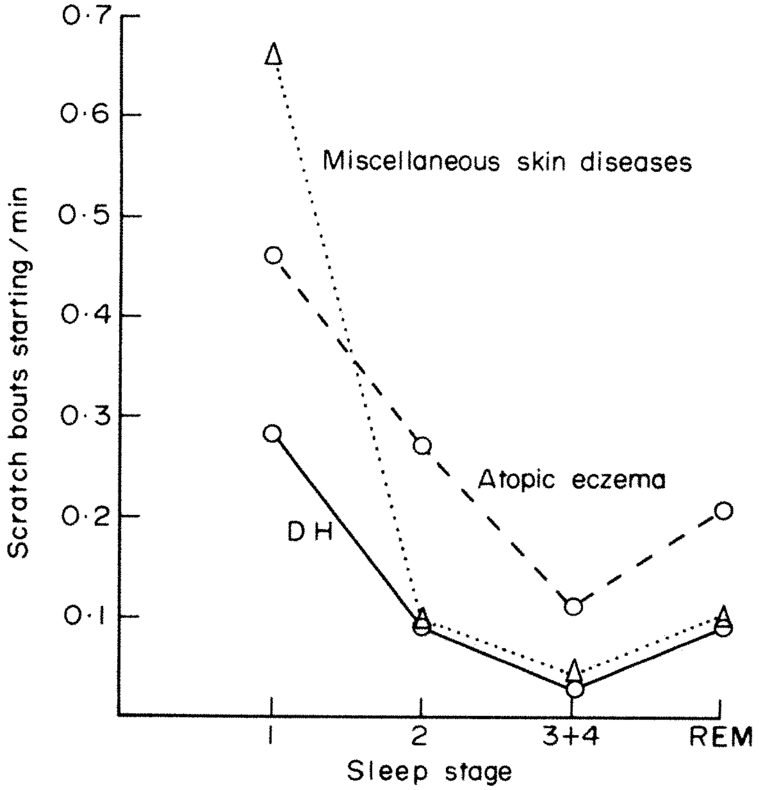

FIGURE 1. The frequency of bouts of scratching starting in each stage of sleep.

TABLE 1. Mean frequency and standard deviation of scratching bouts starting in wakefulness and each stage of sleep in scratches/min

Patients	Sleep stage				
	Awake	1	2	3+4	REM
Atopic eczema (n = 5)	0·47 ± 0·04	0·46 ± 0·40	0·27 ± 0·26	0·11 ± 0·07	0·21 ± 0·22
Dermatitis herpetiformis (n = 5)	0·47 ± 0·08 (n = 3)	0·28 ± 0·11	0·09 ± 0·02	0·03 ± 0·01	0·09 ± 0·06
Miscellaneous group (n = 5)	0·37 ± 0·29	0·66 ± 0·37	0·09 ± 0·12	0·04 ± 0·05	0·10 ± 0·10
Overall means (n = 15)	0·43 ± 0·18 (n = 13)	0·47 ± 0·34	0·15 ± 0·18	0·06 ± 0·06	0·13 ± 0·15

TABLE 2. Mean duration of bouts of scratching starting in wakefulness and in each stage of sleep. Mean ± s.d. (s/bout)

Patients	Sleep stage					
	Awake	1	2	3 + 4	REM	Average 1,2,(3 + 4) & REM
Atopic eczema (n = 5)	17·7 ± 5·6	10·4 ± 3·4	11·5 ± 5·2	12·7 ± 6·0	10·8 ± 5·0	11·4 ± 4·5
Dermatitis herpetiformis (n = 5)	11·5 ± 4·2	8·5 ± 2·9	10·3 ± 4·1	9·6 ± 4·7	7·0 ± 0·6	8·9 ± 2·6
Miscellaneous group (n = 5)	13·8 ± 5·5	12·5 ± 2·0	11·7 ± 4·4	10·0 ± 2·4	14·1 ± 7·8	12·1 ± 3·0
Overall means (n = 15)	14·3 ± 5·4	10·5 ± 3·1	11·2 ± 4·3	10·8 ± 4·5	10·6 ± 5·8	10·8 ± 3·5

variation was high (d.f. = 42·14; $f = 4{\cdot}52$, $P < 0{\cdot}01$), the means for each stage were strikingly similar—approximately 10 s duration per bout.

The significant intersubject variation was analysed further, revealing a characteristic duration of scratching for each person, that is to say a patient who tended to have particularly long bouts of

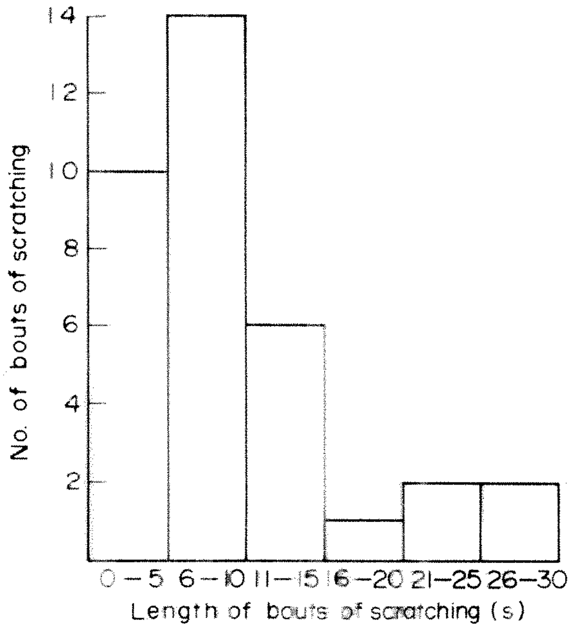

FIGURE 2. The duration (s) of all scratch bouts recorded during stage 1 sleep on a single night of one patient with dermatitis herpetiformis.

TABLE 3. Correlations (τ) and their significance level between different stages of sleep

Durations of scratch bouts in	Stage 1	Stage 2	Stages 3+4	Stage REM
Stage 1		$\tau = 2.69$ $P > 0.01$		
Stage 2			$\tau = 2.55$ $P > 0.05$	$\tau = 2.29$ $P > 0.05$
Stages 3+4				$\tau = 2.29$ $P > 0.05$

scratching during stage 1 sleep would, on average, sustain this lengthy pattern through the other stages of sleep. Kendall's ranking test revealed a correlation between the individual subject's mean duration of bouts in one stage when compared with the other stages of sleep (Table 3).

Individual scratch bouts vary considerably in duration even in the same person in a single stage of sleep (Fig. 2).

Scratching while awake

Bouts of scratching were also recorded when the patients were in bed but awake. There was no significant difference between the frequency of occurrence of scratch bouts during wakefulness and during stage 1 of sleep. However, bouts of scratching when the patients were awake lasted significantly longer on average (14.3 ± 5.4s), than during sleep (10.8 ± 3.5s); $t = 3.77$, d.f. $= 14$, $P < 0.01$.

DISCUSSION

In our previous paper we showed that in atopic eczema bouts of scratching occurred more frequently during sleep stages 1 and 2 than in 3 and 4, with paradoxical sleep in an intermediate position. We have now found this pattern also in patients with other itchy skin diseases such as dermatitis herpetiformis, lichen planus and urticaria (Fig. 1); in other words, this may be a general phenomenon and not one simply confined to atopic eczema.

The nature of the chemical agents causing the itching in these conditions is not well understood; but, in such dissimilar diseases, the agents involved may well not be the same. It seems unlikely that the chemicals involved would vary in the same way in all these diseases during the different stages of sleep. The differences between the frequency with which bouts of scratching occur in these stages can be linked more plausibly to changes in the excitability of the central nervous system than to alterations in the skin lesions themselves. The frequency with which bouts occur during the different stages is consistent with the order of general reactivity of these stages as judged by other criteria; for example, the threshold of response to auditory stimuli (Williams, Morlock & Morlock, 1966), and the level of blood pressure (Coccagna *et al.*, 1971).

In experimental animals there is a spinal reflex pathway for scratching, but in man scratching may be a 'reaction rather than a reflex, demanding the integrity of at least some part of the brain for its performance.' (Sinclair, 1973). We have shown that, while the frequency of bouts of scratching varies among the stages of sleep, the duration of the bouts themselves does not. Bouts of scratching last, on average, just as long whichever stage of sleep they start in. At first sight, this suggests an 'all or none' type of response, in which the size of the reaction is fixed and independent of the stimulus, provided this is over a threshold value. However, Sherrington (1966) demonstrated that this is not the case in experimental animals, in whom the greater the stimulus, the longer and more vigorous is the bout of scratching which follows. Reviewing our figures in this light, it is clear that there is considerable variation in the length of scratch bouts even for one person in a single sleep stage (Fig. 2). It is only the means which are the same. If scratching during sleep is a reflex action, our findings suggest that the excitability of the arc is greatly influenced by the stage of sleep, while the motor side, as judged by the length of the scratch bouts, is not. During wakefulness, bouts of scratching were frequent (Table 1), and lasted significantly longer than during any of the stages of sleep; perhaps a conscious 'deliberate' component had been added.

ACKNOWLEDGMENTS

A Clinical Research Grant was received from the Lothian Health Board and Kirstine Adam was supported by a grant from the Medical Research Council.

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British Journal of Dermatology (1975) **93**, 303.

A new method for the measurement of itch and the response to treatment

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Accepted for publication 28 February 1975

SUMMARY

Two new methods are described for the quantitative measurement of itch: the principle is to measure scratch which is the major objective correlate of itch. The first method is to record whole body movement at night by a vibration transducer on a bed leg; this gives qualitative and quantitative information. The second method is to measure limb movement with movement-sensitive meters; this gives only quantitative data but is quicker and more easily carried out. The subjective sensation of itch was found to correlate well with nocturnal scratch, confirming the basic assumption of the method. Itching as measured by scratch occurs throughout the night and increased severity of itch is accompanied by an increase in the number of bouts of itch and not in their duration. Nocturnal restlessness is associated with an increase in leg movement and so can be dissociated from itch-provoked scratch, which is mostly done by the hands. The response of pruritus to treatment was demonstrated by both methods.

The study of itch and its therapeutic control is bedevilled by the absence of methods for its objective measurement. Since scratch is the main objective correlate of itch we decided to use scratch movement for its measurement. We have devised two methods for measuring scratch movements, one suitable for detailed qualitative and quantitative analysis of itch in individual patients, and the other suitable for quantitative studies in larger numbers of patients. We believe these to be the first quantitative methods devised for the measurement of itch.

METHODS

(1) *Bed movement—using proximity vibration transducers*

Each leg of a standard hospital bed is positioned on a rectangular metal support; a proximity vibration transducer is attached to one of the supports so that any movement by the bed-leg is detected by the transducer. The signal is then amplified and continuously recorded on paper. The patient is completely unaware of the recording apparatus which is in a side-room close to the ward, and body movements are recorded throughout the night whilst the patient is asleep in the ward. For maximum discrimination between the different movements recorded, as well as for economy of paper, we have fitted a device into the circuit such that movement of a pre-selected intensity activates the recorder to run at a faster speed. The recorder runs at 15 mm/min when the patient is at rest and 150 mm/min during movement, the lag of the recording system both for onset and offset being negligible. A disadvantage of the system as it is used at present, is that amplitude of movement cannot be measured in absolute terms because it varies to a small extent with the patient's position in bed; a modification of the apparatus will be used in future studies.

By direct observation of the patient the tracing characteristic of scratching due to itch has been defined; it can easily be distinguished from other types of body movement. This is illustrated in the recordings from a patient shown in Fig. 1. The first part of the recording shows a typical scratch movement by the hand followed by a movement not associated with scratch (turning over in bed). The third part of the tracing is another episode of itch-provoked scratch by the hand superimposed this time on a body movement not due to scratch. A similar tracing is seen with scratch movements by the feet. The characteristics of scratch movement tracings are the regularity of the peaks and troughs representing individual scratch movements with a frequency in the order of 2/s: the movements are symmetrical about the baseline which does not move. By contrast other movements displace the baseline; they are irregular, their amplitude is much greater than scratch movements and their frequency less. Thus the method allows analysis of type of movement throughout the night and its quantification as duration, amplitude and frequency.

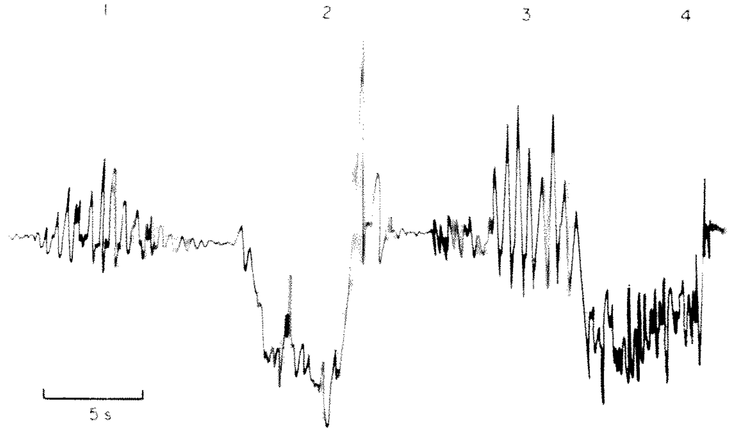

FIGURE 1. Tracing of nocturnal movements using proximity vibration transducer. 1 and 3 show pure scratching movement, 2 shows non-scratch movement (turning in bed), 4 shows a similar movement with a superimposed scratch movement.

(2) *Limb meters*

We have measured the movement of individual limbs by movement-sensitive meters. Each meter is a 'self-winding' watch in which the mechanism has been so modified that movement of the watch is registered directly by movement of the hands and calendar dial. The meters are secured to the wrists and ankles by rubber straps with a 'Velcro' fastener. The lightness of the meters and their attachment are such that they cause no disturbance and patients seem to be oblivious of their presence. When the patient decides to retire at night the position of the two hands on the meter and the number on the calendar dial are recorded. The meters are removed immediately on awakening and the movement

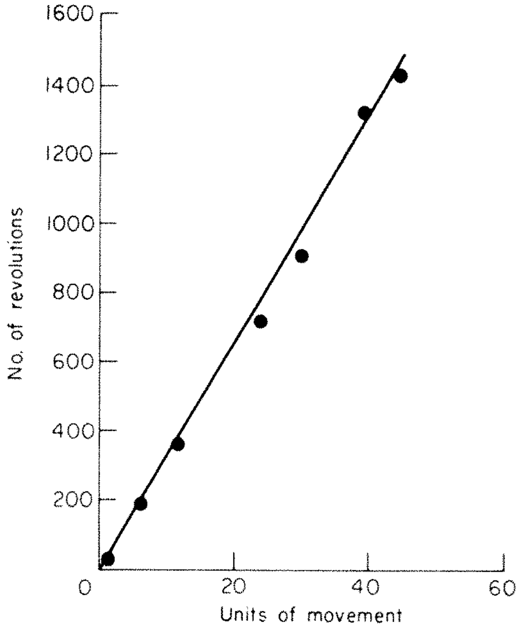

FIGURE 2. Calibration of limb meters on turntable.

recorded as the increase in units on the dial. These units were then referred to a calibration curve which was constructed for each of the 21 meters used.

For calibration, each of the meters was attached to a turn-table rotating at a constant speed. A linear relationship was found between movement (number of revolutions of the rotating table Fig. 2) and the movement recorded demonstrating that the meters give an accurate measurement of movement in the range used. The sensitivity of each meter was found to be virtually identical and regular calibration of the meters against one which was kept solely for calibration revealed no alteration in their sensitivities over a period of 30 months.

RESULTS

Bed vibration transducers

Nocturnal movements were studied from 22.30 to 06.00 h in eight patients with itch due to eczema. The results were similar in each of the eight patients and are illustrated for one, a patient with only moderate itching, before and during treatment with topical corticosteroids (Fig. 3). All movements were analysed throughout each of the 5 nights of the study and separated into scratch movements and non-scratch movements. Scratch movements were further analysed into the total number of bouts of scratching, total scratch time and the mean duration of each bout of scratching. Before treatment, bouts of scratching occurred throughout the night on average every 11·7 min, each bout lasting an average of 0·35 min. There was considerable difference between individuals and on some nights total scratch time occupied as much as 11% of sleeping time. As the patient's sensation of itch decreased with treatment so did scratch. This decrease in total scratch time was due to an overall reduction in the number of bouts of scratching; interestingly there was no change in the mean duration of bouts of scratching or in the frequency of scratch movements within a bout (Fig. 3). The amplitude of scratch

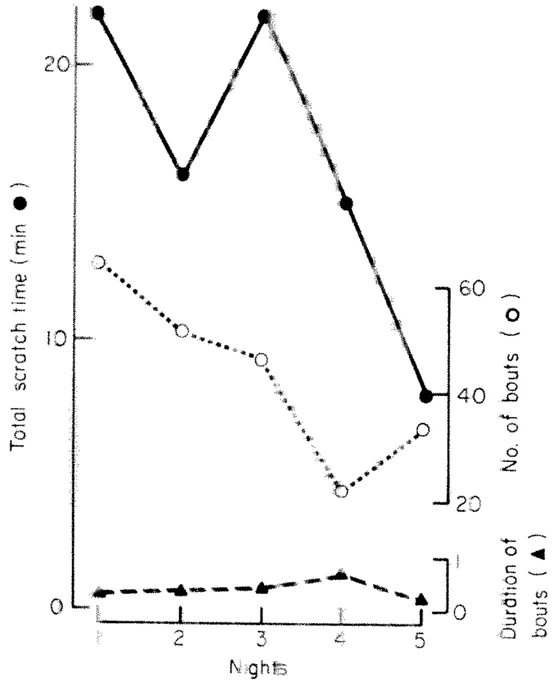

FIGURE 3. Analysis of scratch movement during treatment in a patient with eczema.

movements did not decrease but because this measurement is position sensitive with the present system, this point will require verification.

Limb meters

Seventy-seven male and female patients aged from 15 to 82 years were studied. Fifty-six patients had been admitted for eczema, dermatitis herpetiformis, lichen planus, psoriasis or scabies; the re-

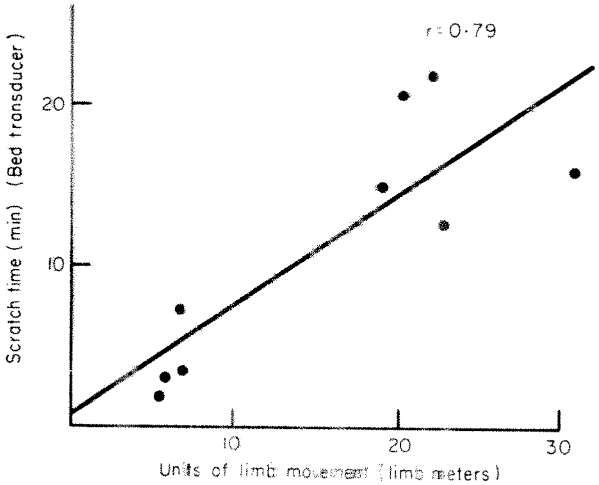

FIGURE 4. Correlation of nocturnal scratch movement (as scratch time) measured by the bed transducer with limb movement measured by meters.

maining twenty-one patients had been admitted for investigation and treatment of various non-itchy conditions such as collagen vascular disorders, and served as controls. Limb movements were measured between 22·30 and 06·00 h on the first and second nights of admission in all seventy-seven patients. None of the patients was taking drugs or using applications other than emulsifying ointment (NF) on these 2 nights. Further measurements were made on the subsequent 3 nights in the control group and the subsequent 3–13 nights in the twenty-five patients with eczema in whom the response to topical therapy with betamethasone valerate or clobetasol was being studied in detail.

(a) *Validation of method*

(1) *The measurement of scratch.* Simultaneous measurements of nocturnal limb movements by limb meters and pure scratch movements by the bed transducer were carried out on 3 consecutive nights in three patients with itch due to eczema. The results (Fig. 4) show an excellent correlation between nocturnal limb movements measured by the meters and actual scratch movement ($r = 0{\cdot}88$). Thus nocturnal limb movement as measured by the meter is a satisfactory measure of scratch.

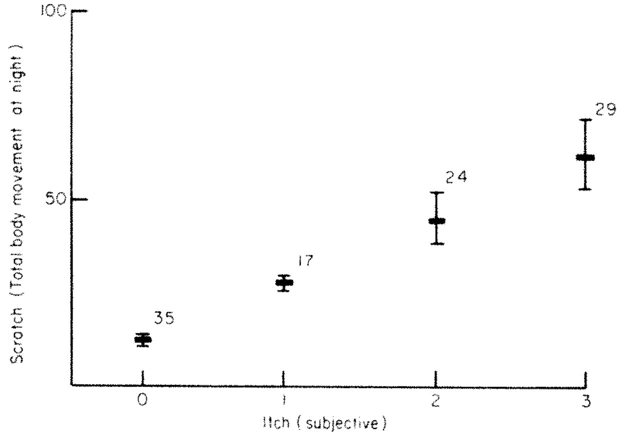

FIGURE 5. Correlation between the subjective sensation of itch and the measurement of nocturnal scratch using limb meters in patients with various itchy dermatoses.

(2) *The correlation of scratch with itch.* Using the limb-meters scratch was measured as nocturnal limb movements on the first 2 nights of admission in the fifty-six patients with eczema, dermatitis herpetiformis, lichen planus, psoriasis or scabies. The patient's subjective sensation of itch was graded independently on a 0–3 scale (nil, mild, moderate, severe). A good correlation was found between itch and scratch (Fig. 5) indicating that itch can be measured as scratch using limb meters.

(b) *Analysis of nocturnal movement due to itch and restlessness*

Limb movements were measured in the twenty-five itchy patients with eczema and the twenty-one control patients without itchy dermatoses and without itch. The itchy patients had an appreciable increase in limb movements (Fig. 6). Amongst the non-itchy controls, however, there was a clearly identifiable subgroup who were observed to be restless at night and who admitted to restlessness when questioned. This group could also be separated by analysis of their limb movements: they showed an increase in total limb movement and this was found to be due to leg movement. Thus restlessness can easily be dissociated from itch-induced scratch by the arm/leg movement ratio (Fig. 7). The separation of itchy and restless patients by the measurement of arm and leg movements was also confirmed by the

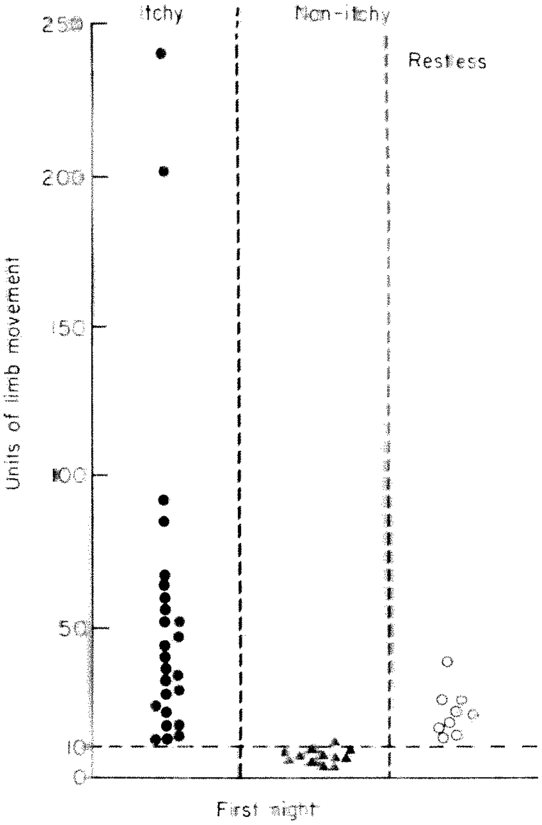

FIGURE 6. Nocturnal limb movement in itchy patients with eczema and in non-itchy control subjects. Restless subjects formed a clear subgroup of the non-itchy individuals.

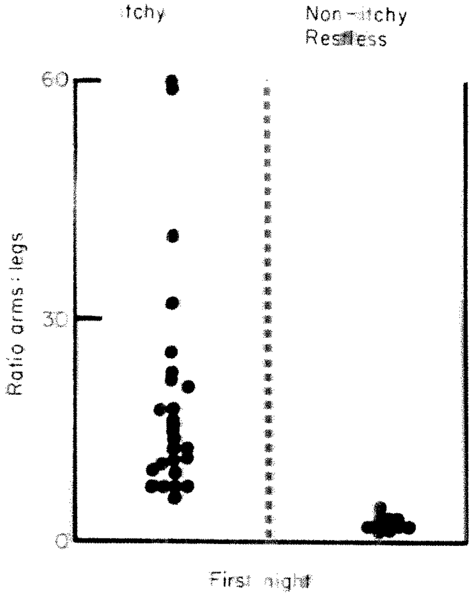

FIGURE 7. Ratio of arm movement to leg movement in itchy and restless patients shown in Fig. 6.

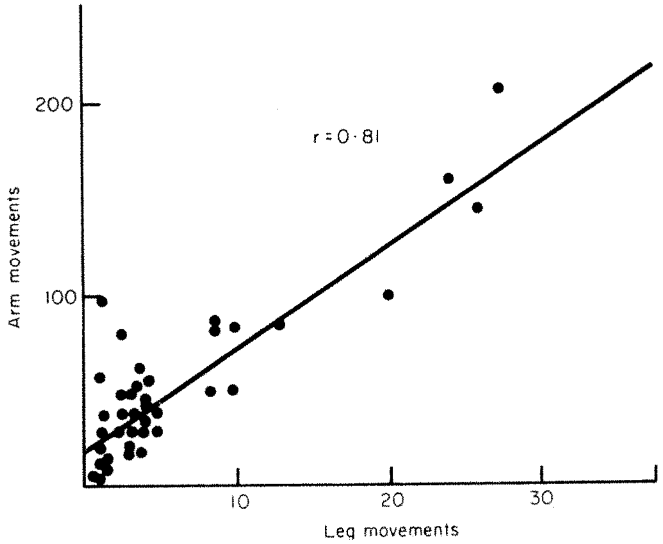

FIGURE 8. Arm movement compared with leg movement on the 1st and 2nd nights of admission in the itchy patients shown in Fig. 6.

simultaneous use of limb meters and the bed vibration transducer (to be reported). Taking leg movement as a measure of restlessness it is apparent that itch provokes a degree of restlessness (Fig. 8). This was also apparent from the bed transducer tracings, the restless movement decreasing with treatment (Fig. 9).

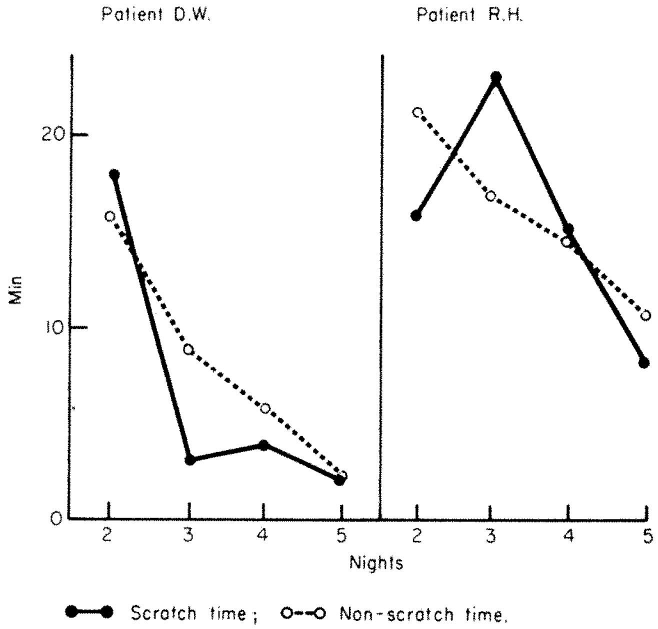

FIGURE 9. Effect of treatment on scratch movement and non-scratch movement in two patients with eczema using the bed transducer.

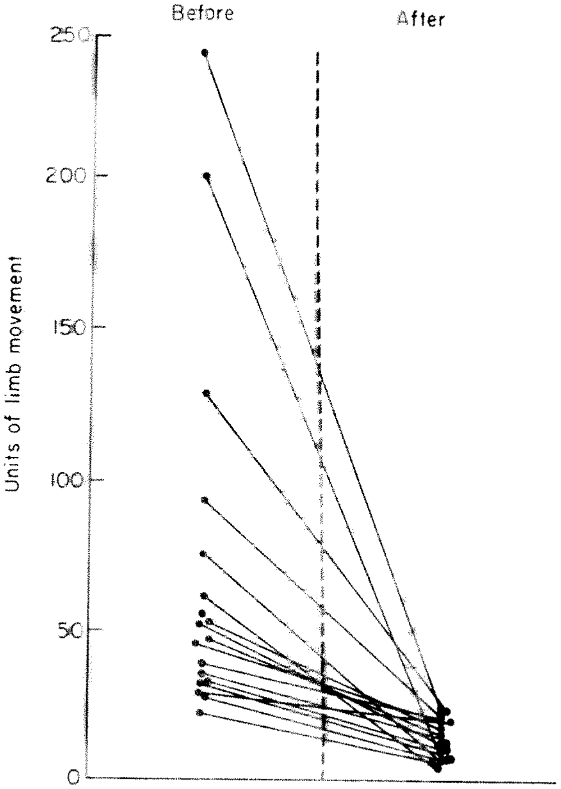

FIGURE 10. Effect of treatment on itch-provoked scratch measured by limb meters in eighteen patients with eczema.

(c) *The effect of treatment*

Using the limb meters we measured itch on admission and 1–2 weeks later, after successful treatment of the eczema with a topical corticosteroid. The sensation of itch disappeared and limb movements decreased towards the normal nocturnal level (Fig. 10).

DISCUSSION

Direct non-subjective measurements of a sensation cannot be made. The principle which we have adopted is to measure an objective correlate of the sensation and in the case of itch the correlate is scratch. Itch can be defined as a sensation which provokes the desire to scratch; indeed it is not improbable that the biological purpose of itch was to induce scratching and thereby to remove parasites. Most skin parasites live very superficially in the skin and the itch receptors appear to be very superficially organized pain fibres (Keele & Armstrong, 1964); thus itching stops either when the sensation is eased or when it is replaced by pain, at which state the superficial layers of the skin, and with them the itch receptors and the parasites, will have been removed by scratching. The present findings of a direct correlation between the subjective sensation of itch and the objective measurement of scratch supports these views and validates the measurement of itch by scratch. The slope of this relationship does however vary in different clinical situations and this may in part be related to the central and peripheral components of the itch sensation (in preparation).

In the present study we have presented data on nocturnal scratch because of the complication which is introduced during the waking hours by movements which are not due to scratch. The methods we have described can however be used to study itch-provoked scratch by day, and we have found that the results by day correlate surprisingly well with those obtained from the same patients at night.

The two methods used have quite different features and can be used for different purposes. The measurement of whole body movement as bed vibration gives both qualitative and quantitative information: the time at which scratching occurs together with the number of bouts of scratching and their duration, amplitude and frequency can be measured. Furthermore the method has the advantage that the patient can be completely unaware that his nocturnal movements are being monitored whilst in an ordinary hospital bed in an open ward under normal hospital conditions. The disadvantage of the method is that it is too cumbersome and tedious for large scale studies such as are required for example with the comparison of antipruritic drugs. Using this method we have shown that scratching is done mainly by the hands and occurs in bouts throughout the night. A greater itch produces more frequent bouts of scratching rather than an increase in duration of the bouts. It would be interesting to correlate these nocturnal movements with sleep and EEG changes (Savin, Paterson & Oswald, 1973).

It appears that very itchy patients may spend more than 10% of the night scratching. In addition itch provokes a degree of restless movement. Thus limb meters worn by night and day show that itchy patients may make as much as 40% of their total daily (24 h) movement during their 7·5 h of 'sleep'. The energy involved in scratching must be considerable and this may be a factor in the very high metabolic rates which have previously been observed in patients with skin disease (Shuster & Marks, 1970).

The measurement of limb movements with meters is much simpler than the measurement of total body movement with proximity vibration transducers, but the meters do not qualitatively separate itch-provoked scratch from restlessness. However, by using limb meters and the vibration transducer simultaneously we found that the increased limb movements of restless patients were done mainly by their legs whilst itchy patients used their arms to scratch. Thus restlessness and itch provoked scratch can be separated by using arm and leg meters simultaneously. In this way we have found that a surprisingly large number of patients admitted to a skin ward for non-itchy dermatoses are restless at night with an increase in leg movements and a low arm to leg movement ratio. Using limb meters it is therefore possible to separate itchy, normal and restless subjects, and the movements are such that there is little or no overlap between the three groups. The findings with the limb meters, as with the bed transducer, show that scratching is done predominantly by the hands, and the sheen which can be observed on the nails of patients who itch highlights the action used.

The simplicity and quantitative nature of the methods described are ideal for the study of the physiopathology of itch, and, of particular importance to the dermatologist, for the selection of optimal therapeutic regimens (Felix & Shuster, in preparation). The methods could also be used to study aspects of normal sleep and body movement, and the effect of drugs upon them.

ACKNOWLEDGMENTS

We are particularly grateful to Dr Heinz Wolff and Mr Fogarasy for advice and help with the limb meters and vibration transducers and Mr Jim Gossop and Mr Lawrence Chadwick for their able technical assistance.

We wish to acknowledge a grant from the Medical Research Council and from the Research Committee of the Royal Victoria Infirmary.

British Journal of Dermatology (1975) **93**, 313.

Coexistence of lichen planus and bullous pemphigoid

AN IMMUNOPATHOLOGICAL STUDY

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Accepted for publication 16 December 1974

SUMMARY

A 43-year-old white man presented with a generalized eruption of lichen planus and tense blisters within the lichenoid lesions and also on clinically normal skin. Direct immunofluorescence (IF) studies revealed immunological and histopathological characteristics of lichen planus in the lichenoid lesions and of bullous pemphigoid in the bullous lesions, and indirect IF studies showed that the patient had circulating antibasement membrane antibodies. The coexistence of both disorders may indicate a possible link between the pathology in the junctional zone in lichen planus and the appearance of antibasement membrane zone antibodies and bullous lesions, respectively.

Immunofluorescence (IF) techniques are an established tool in the differential diagnosis of bullous dermatoses (Beutner, Chorzelski & Jordon, 1970) and in lupus erythematosus (LE) (Jablonska & Chorzelski, 1974). Moreover, these methods permit insight into the pathogenetic mechanisms operative in these diseases. Characteristic findings have also been reported in several other disorders, e.g. lichen planus (LP) and vasculitis, but in these diseases their significance is still open to discussion. As regards LP, only direct IF investigations have revealed positive results, with clusters of cytoid bodies in the papillary dermis and in the junction zone, demonstrable with antisera to immunoglobulins (Ig) G and M, occasionally also to IgA and to (C_3 (Michel *et al.*, 1970; Ueki, 1969). In addition, massive deposits of fibrin and fibrinogen were found to outline the basement membrane zone (BMZ). In this paper we describe a patient who presented with typical lesions of lichen planus and a bullous eruption indistinguishable from bullous pemphigoid. Immunologically, there were features of both diseases.

CASE REPORT

A 43-year-old white man with a 6-months history of itching papules and blisters first presented at our outpatient clinic in November 1973. Topical therapy with steroid-containing ointments had not

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* Supported, in part, by Schering AG, Berlin.

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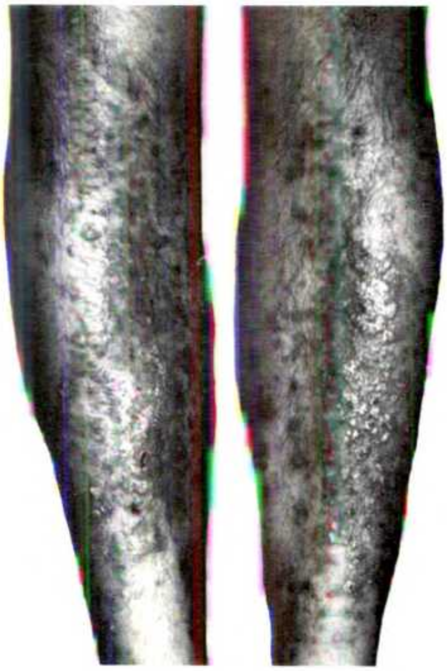

FIGURE 1. Typical LP lesion over the shins with tendency to coalescence and plaque formation.

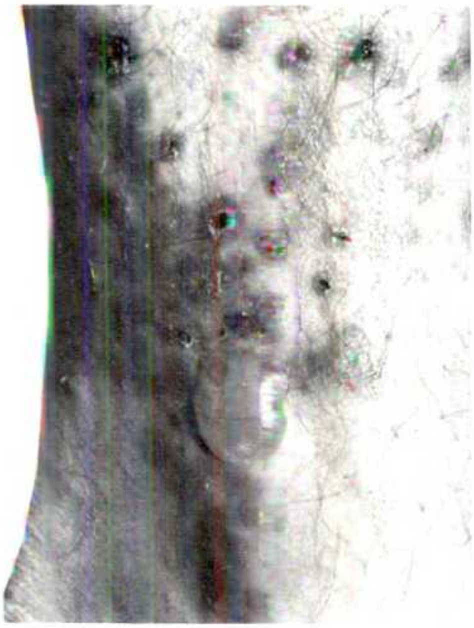

FIGURE 2. Several tense blisters up to the size of a pea adjoining an LP plaque on the right supramalleolar region.

prevented the spreading of LP lesions and blisters. On admission (November 1973) he presented with a generalized eruption of typical LP papules which tended to coalesce and form infiltrated plaques particularly over the shins (Fig. 1). Within these plaques several tense, pea-sized blisters containing serous fluid were observed (Fig. 2). Similar lesions were found on the arms, chest, abdomen and sacral region. In the sternal area and on the inner aspect of both thighs, blisters up to the size of a walnut were observed also on clinically normal skin. The buccal mucosa exhibited the classical features of LP with a network of white streaks and patches.

Investigations and treatment

The following were within normal limits: full blood count, urinalysis, fasting blood sugar, serum glutamate pyruvate and glutamate oxalacetic acid transaminases, bromsulphthalein-test, immunoglobulin levels of the serum, blood urea, serum creatinine and creatinine clearance. Thyroid function was checked because of slight exophthalmos but was normal.

Treatment was instituted with 30 mg of fluocortolone daily, gradually reduced every second day to 10 mg, together with 150 mg azathioprine per day.

With this regimen the lesions both of lichen planus and of bullous pemphigoid type improved rapidly within 6 weeks. There was no difference in the rate of clearing of bullous and of lichenoid lesions. Subsequently, the patient has been discharged from the hospital free of skin symptoms. Except for a few occasional lichenoid papules or vesicles he remained free from January to September 1974.

Histopathology

A specimen taken from a lichen planus papule revealed the typical picture with hyperkeratosis, hypergranulosis, sawtooth-like appearance of rete pegs and a dense lymphocytic infiltration of the papillary dermis (Fig. 3). A specimen from a bullous lesion exhibited a subepidermal blister with only a sparse cellular infiltrate in the dermis (Fig. 4). A third specimen taken from the buccal mucosa showed changes typical of lichen planus.

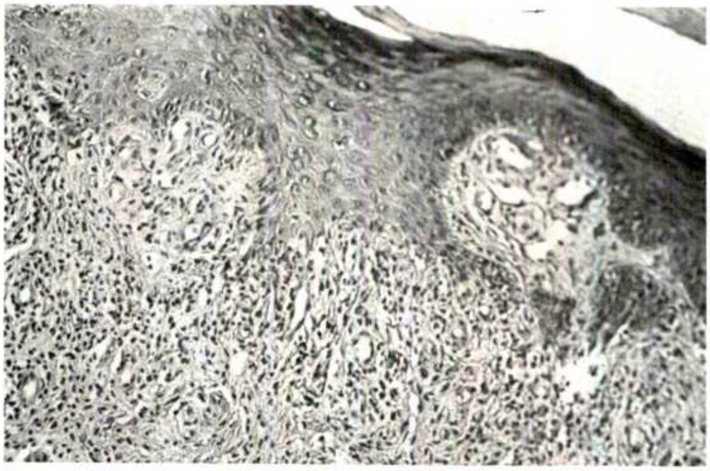

FIGURE 3. Histopathology of an LP lesion. Note hyperkeratosis, hypergranulosis, sawtooth-like appearance of rete pegs, and the dense lymphocytic infiltration of the dermis.

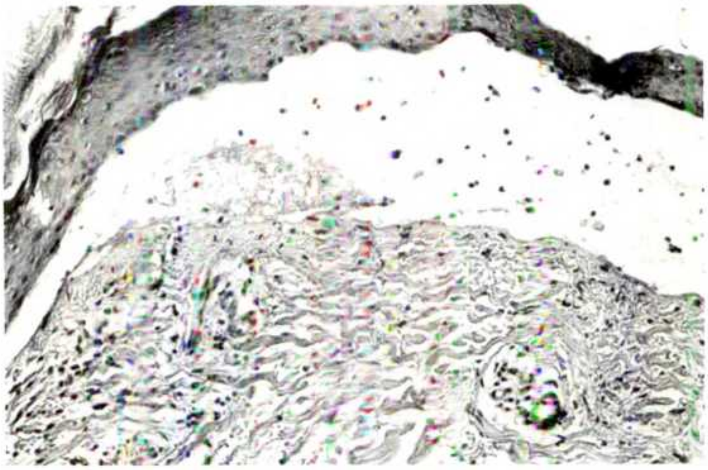

FIGURE 4. Histopathology of a bullous lesion. Note the subepidermal blister, containing several inflammatory cells and fibrin, and the sparse mononuclear infiltrate in the dermis.

Immunofluorescence studies

Skin specimens used in the direct IF studies were obtained by excision, were quick frozen in liquid nitrogen and stored at −20°C. Sections were cut in a cryostat at 4–6 μm and examined within 3 days. Rhesus monkey oesophagus served as a substrate for indirect IF tests. Initial serum dilutions were 1/10, phosphate buffered saline (PBS)—4% bovine serum albumin (BSA), pH 7·2, being used for dilution. Commercially prepared fluorescein isothiocyanate (FITC) conjugated goat-antisera to fibrin, fibrinogen, and C_3 were utilized. IgG, IgA and IgM antisera, of goat origin, were kindly provided by Dr E.H. Beutner, Buffalo. Antibody (ab) levels were checked with reverse immuno-diffusion (RID) plates kindly provided by Hyland, Inc., Costa Mesa, California, USA.

Characteristics of the antisera were as follows: IgG antiserum, FITC 61·9 μg/ml, total protein 8·7 mg/ml; F/P (weight) $7{\cdot}11 \times 10^{-3}$ or 2·9 (molar), specific antibody 2·6 mg/ml diluted to 50 μg ab/ml; IgM antiserum, FITC 48·5 μg/ml, total protein 10·28 mg/ml; F/P (weight) $4{\cdot}72 \times 10^{-3}$ or 1·92 (molar), 2 mg ab/ml diluted to 50 μg ab/ml; IgA antiserum, FITC 75 μg/ml, total protein 14·5 mg/ml; F/P (weight) $5{\cdot}17 \times 10^{-3}$ or 2·12 (molar), specific antibody 470 μg/ml diluted to 100 μg/ml.

All determinations of total protein values were performed before any absorption procedures. Antibody values of antisera to fibrin, fibrinogen and C_3 could not be reassayed with RID plates and working dilutions were determined by 'titrations' on sections from biopsies of LP and bullous pemphigoid lesions, respectively. Dilutions (in PBS–4% BSA) used were 1/16, 1/16 and 1/32, respectively. Photomicrographs were taken on Kodak Ektachrome high speed color slide film at 400 SAE sensitivity position of a Zeiss photomicroscope II, equipped with a Tiyoda superwide dark field condenser. Filter equipment was: excitation filter KP 490 (2 ×) (interference filter, Schott und Gen., Mainz, Germany). Barrier filter Zeiss 50.

Results of IF-tests

Circulating BMZ antibodies were detected with the indirect IF-staining method (Fig. 5). At the time of admission of the patient the titre was more than 320 but dropped to 40 under the corticosteroid–azathioprine treatment. The blister fluid yielded BMZ ab in titres of 20–40. By using the direct

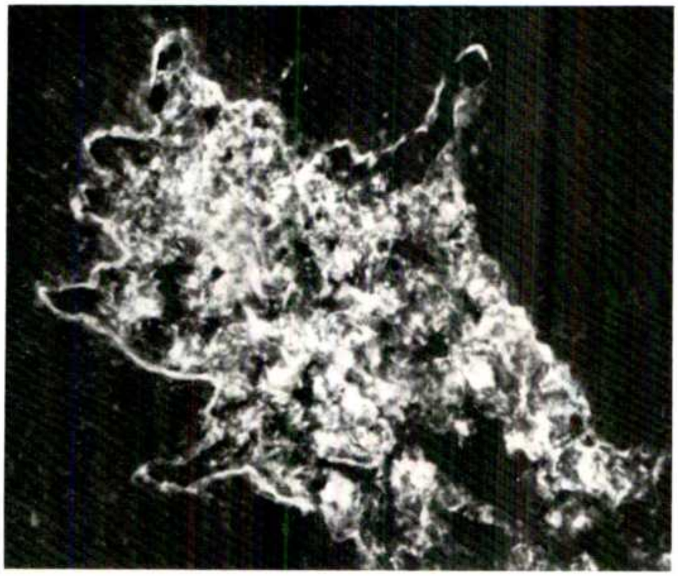

FIGURE 5. Demonstration of circulating BMZ-antibodies with the indirect IF-staining technique. A goat anti-IgG conjugate was used, the initial serum dilution was 1/10. (Magnification × 40.) (For details see under 'Immunofluorescence Studies'.)

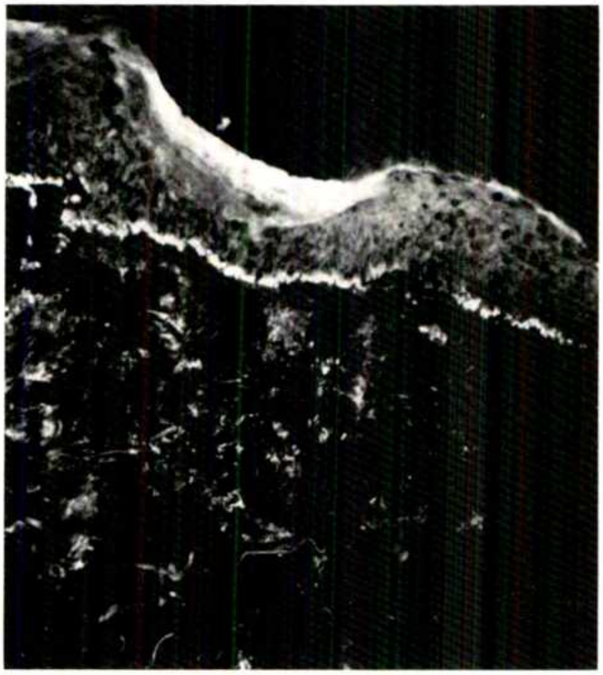

FIGURE 6. Positive direct IF with IgG antiserum (goat origin). BMZ-pattern within a bullous lesion. (Magnification × 40.) (For details see under 'Immunofluorescence Studies'.)

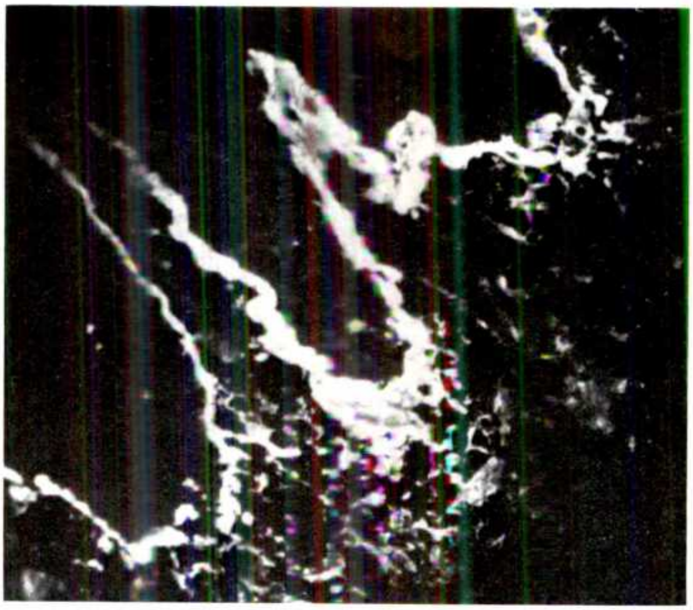

FIGURE 7. Positive direct IF with fibrin-antiserum. Note the heavy band-like deposits of fibrin within a LP lesion. (Magnification × 100.) (For details see under 'Immunofluorescence Studies'.)

IF-staining method a conspicuous fluorescence along the BMZ was seen in the bullous lesions with anti-IgG- (Fig. 6), anti-IgM-, and anti-C_3-conjugates. Only a few cytoid bodies were observed but fibrin or fibrinogen was not detected except within the blister cavity.

In contrast, the LP lesions exhibited heavy band-like deposits of fibrin and fibrinogen (Fig. 7) along the BMZ and some clusters of cytoid bodies containing IgG (Fig. 8) and C_3.

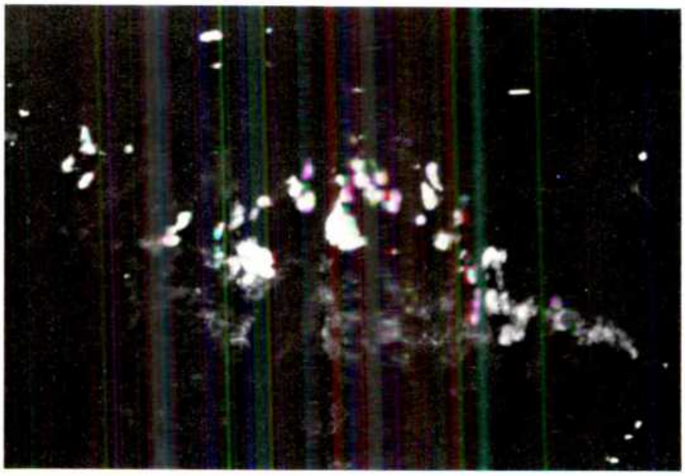

FIGURE 8. Positive direct IF with IgG antiserum. Clusters of cytoid bodies are seen within the papillary dermis. (For details see under 'Immunofluorescence Studies'.)

DISCUSSION

An increasing number of cases with a coexistence of autoimmune diseases has been reported within the last few years. There are reports of concurrence of pemphigus with myasthenia gravis with and without thymoma (Beutner *et al.*, 1968; Peck *et al.*, 1968), and most recently, of pemphigus and bullous pemphigoid (BP) (Chorzelski *et al.*, 1974). Some authors interpret pemphigus erythematosus as reflecting a coexistence of pemphigus and LE (Bean & Lynch, 1970; Chorzelski, Jablonska & Blaszczyk, 1968; Orfanos, Gartmann & Mahrle, 1971). As regards LE (Alarcon-Segovia *et al.*, 1963; Davis, 1966; Denney & Rose, 1961; Larsson, 1963; Peck *et al.*, 1968; Wolf & Burrows, 1966) there is also some evidence of a transition into, or concurrence with, other auto-immune phenomena. The same holds for bullous pemphigoid, e.g. coexistence of BP and LE (Jordon *et al.*, 1969), BP and ulcerative colitis (Bean, Good & Windhorst, 1970), BP and rheumatoid arthritis (Lillicrap, 1963), and BP and polymyositis (Peck & Lefkovits, 1966). The possibility of a basic immune disturbance underlying two or more concomitant auto-immune diseases has been suggested. As far as LP is concerned there are few reports of an association with auto-immune diseases, e.g. reports in which a differentiation between LP and LE has not been possible on clinical, histological or immunological grounds (Copeman, Schroeter & Kierland, 1970; Jablonska & Chorzelski, 1974). To our knowledge, the case presented in this paper appears to be the first in which there is histological and immunological evidence for the coexistence of LP and BP. The diagnosis of either disease seems to be well established. The typical clinical lesions, the characteristic histopathology and the fibrin and fibrinogen deposits along the BMZ together with the observation of clusters of cytoid bodies confirm the diagnosis LP. On the other hand, the diagnosis BP rests on the histopathology and IF-studies of other lesions which revealed *in vivo* deposits of IgG and complement in the BMZ without any conspicuous deposits of fibrin; in addition, the detection of circulating anti-BMZ antibodies makes a good case for the diagnosis of BP. It is entirely possible that our patient reflects a chance association of two independent diseases; but our observations may also be interpreted in an entirely different manner. An immune complex disease that leads to the deposition of Ig over long periods of time may result in a damage of those structures where these complexes are deposited, such as the BMZ (Klassen *et al.*, 1974). Unmasking of hitherto unexposed, or creation of new antigens may ensue and eventually lead to the production of (humoral) autoantibodies reacting with these antigens. Since both the deposition of fibrin and fibrinogen and the observation of cytoid bodies in the BMZ of LP lesions may serve as evidence for such a process, it would not be unreasonable to expect a subsequent development of anti-BMZ-antibodies, the *in vivo* deposition of Ig along the BMZ, and clinically a bullous pemphigoid (like) syndrome.

It could even be speculated that a transformation of any classical lichenoid into a bullous variant of LP might be the consequence of such a BMZ damage. To our knowledge, there have been no previous immunofluorescence studies of patients with bullous LP but it is feasible that findings as were present in our patient may be common in this variant of the disease. Consequently, particular attention should be given to the course of severe cases of LP, especially if bullous lesions occur. Longitudinal studies of cases of LP are under way in our laboratory at this time.

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A prospective study of the effects of weekly oral methotrexate on liver biopsy

FINDINGS IN SEVERE PSORIASIS

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Accepted for publication 6 December 1974

SUMMARY

The results are reported of a prospective study of liver histological findings in twenty-five patients with severe psoriasis subjected to liver biopsy before and at intervals after the onset of treatment with methotrexate by an intermittent dosage schedule. The pre-methotrexate liver biopsy findings in forty-one other severe psoriatics are also summarized.

Of the twenty-five treated patients, four showed a mild inflammatory cell infiltrate and two mild fibrosis before treatment. During treatment only one developed a grade 2 infiltrate and one other grade 2 fibrosis. None has developed cirrhosis.

Where minor abnormalities developed, their fluctuating and reversible nature is demonstrated.

It is established that methotrexate (MTX) can produce hepatic fibrosis and cirrhosis. Fibrosis was first described in 1955 (Colsky, Greenspan & Warren) in children treated for leukaemia. In the late sixties, reports suggested that MTX given to patients with psoriasis could produce fibrosis and cirrhosis (Coe & Bull, 1968; Epstein & Croft, 1969; Muller, Farrow & Martalock, 1969). This led to a number of retrospective surveys, from which it emerged that daily oral treatment was apparently associated with the development of fibrosis and cirrhosis in a few patients (Dubin & Harrell, 1970; Dahl, Gregory & Scheuer, 1971, 1972; Almeyda *et al.*, 1972; Weinstein *et al.*, 1973; Tobias & Auerbach, 1973; Millward-Sadler & Ryan, 1974). A high alcoholic intake, obesity especially associated with diabetes mellitus, increasing cumulative dose of MTX and severity of psoriasis have been suggested as possible predisposing factors (Roenigk *et al.*, 1971; Zachariae & Schiodt, 1971; Zachariae & Sogaard, 1973; Weinstein *et al.*, 1973). These findings emphasize the need for prospective studies of MTX toxicity in patients needing the drug whose liver function and structure have been thoroughly investigated before starting treatment. Other studies are in progress in several countries and we, here, present our own prospective findings to date. We previously published our (mainly retrospective) findings in 1972 (Almeyda *et al.*).

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PATIENTS AND METHODS

All patients had severe and disabling psoriasis and required systemic treatment for its control. All had liver function tests and liver biopsies prior to starting MTX therapy and approximately annually thereafter. Liver function tests will not be discussed in this paper. All biopsies were read by one of us (JWL) without reference to details of MTX dosage. Most patients had only single weekly dose schedules, although five patients had some of their treatment in divided doses over 24 hours each week, and two patients had all their MTX by this regimen. Sixty-six patients had liver biopsies in anticipation of needing MTX and twenty-five of those (11 females; 14 males) had further biopsies after MTX therapy was started. Some of the remaining forty-one patients were not subsequently given MTX for various reasons and the remainder have not yet had a second biopsy.

The ages in the group of twenty-five treated patients ranged from 23 to 78 years (mean = 53 years). MTX has been given to date for 12 to 60 months (mean = 30 months). The cumulative dose of MTX varied from 0·61 to 4·25 g (mean = 1·76 g). The dose never exceeded 0·4 mg per kg body weight per week.

Liver biopsies were taken at least 5 days after a dose of MTX. Fatty change, inflammatory cell infiltrate and fibrosis were each graded on a scale as shown in Table 1.

TABLE 1. Grading of histological abnormalities in the liver

Grade	Non-specific		Fibrosis	Cirrhosis
	Fat	Inflammatory cells		
0	<100 cells per lobule	<50 per portal tract	None	
½ = very mild	Findings just short of grade 1			Not graded
1 = mild	>100 cells to ⅓ lobule	>50 cells per portal tract	Sparse intralobular	
2 = moderate	⅓–⅔ lobule	>100 cells per portal tract	Just bridging portal tracts	
3 = severe	⅔ lobule	>250 cells per portal tract	Bridging portal tracts	

Cirrhosis was deemed to be present or absent. Fatty change was very variable and could not be correlated with other histological changes. The minor degrees of cellular infiltration or fibrosis seen occurred with equal frequency in patients with fatty livers and in those in whom liver biopsies showed little or no fat. In addition, post-MTX biopsies did not show more or less fat than pre-MTX ones. For these reasons, fatty change was not considered to be a reliable index of liver damage due to MTX. The inflammatory cell infiltrate was composed of lymphocytes, plasma cells and small numbers of histiocytes.

RESULTS

The findings in the twenty-five patients who had liver biopsies before and after MTX treatment are shown in Figs. 1 and 2. The inflammatory cell infiltrate is shown in Fig. 1 and fibrosis in Fig. 2. The findings *at each biopsy* are plotted against the cumulative dose of MTX. The graphs show that there is no tendency to progressive hepatopathy.

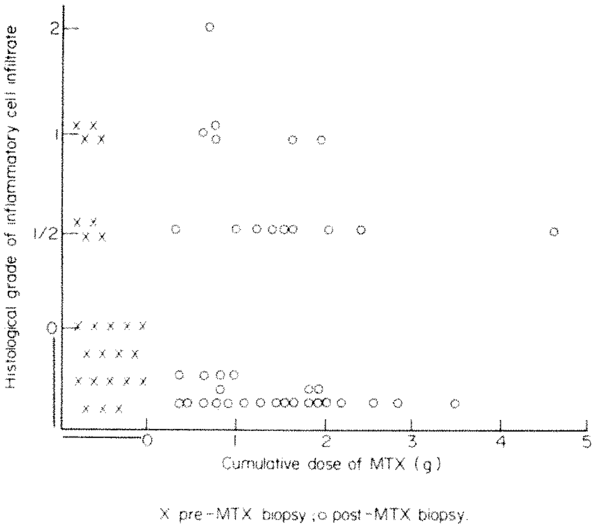

FIGURE 1. Liver biopsies in patients treated with weekly methotrexate: inflammatory cell infiltrate

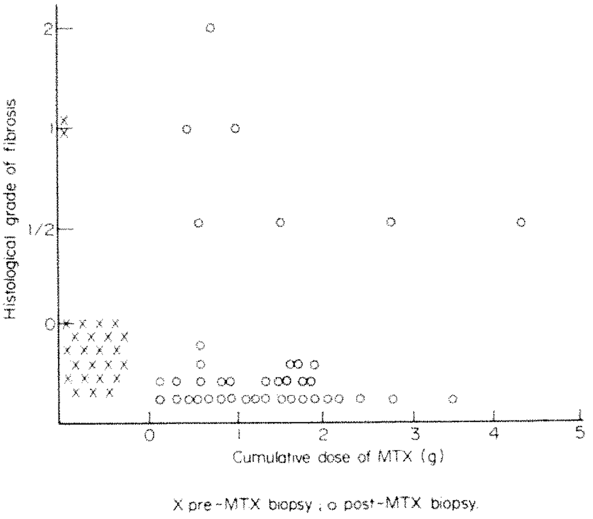

FIGURE 2. Liver biopsies in patients treated with weekly methotrexate: fibrosis.

The degree of correlation between inflammatory cells and fibrosis both before and after MTX is given in Table 2. Fibrosis was usually associated with cellular infiltrate, and in only eight biopsies occurred on its own.

A number of individual patients who did show changes are of interest and are described below.

Mrs S.H. Aged 24 years. (Fig. 3.) Her pre-MTX liver biopsy was normal but she showed grade half fibrosis on her third and fifth biopsies in the absence of increased cellular infiltrate. These biopsy findings are very mild and of doubtful significance. MTX cannot be avoided and a further biopsy will be made 2 years after the last one.

TABLE 2. Correlation between cells and fibrosis in liver biopsies

Completely normal	= 114
Cells increased without fibrosis	= 35
Fibrosis without cells increased	= 8
Cells increased plus fibrosis	= 35
Total biopsies	= 192

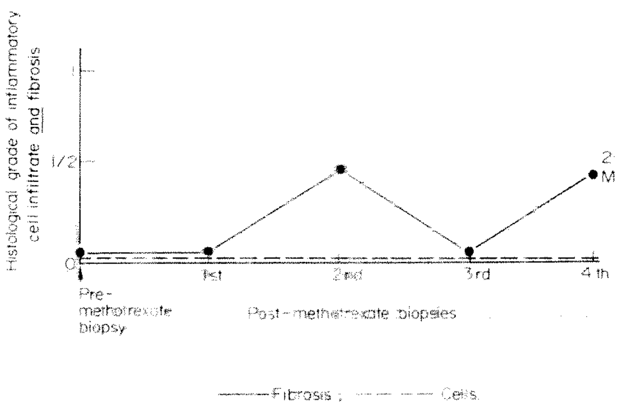

FIGURE 3. Serial liver biopsies in Mrs S.H.

Mr D.B. Aged 53 years. (Fig. 4.) His pre-MTX biopsy was normal and he showed grade 1 inflammatory cells and fibrosis in his first post-MTX biopsy. The drug was nevertheless continued and over the next two biopsies, the liver apparently became normal. On his fourth post-MTX biopsy after a total of 4·25 g MTX he had grade half cells and fibrosis. MTX was then stopped, probably unwisely with subsequent severe relapse of the psoriasis and several periods of prolonged hospitalization and disability since. It is likely that MTX will have to be resumed.

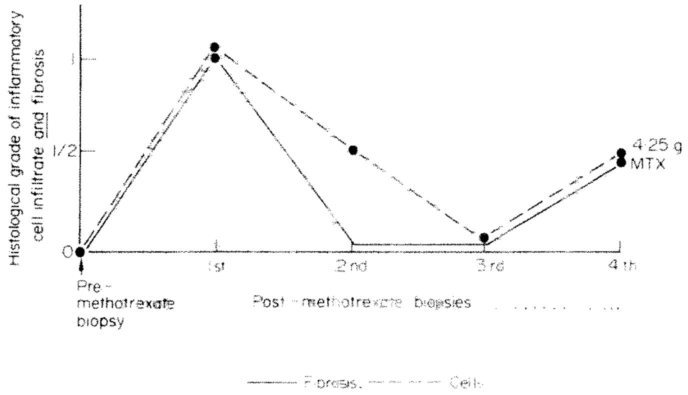

FIGURE 4. Serial liver biopsies in Mr D.B.

Mrs L.M. Aged 29 years. She had a normal pre-MTX biopsy but after 1·0 g of MTX she showed grade 1 fibrosis and grade half inflammatory cell infiltrate. It was decided to stop the MTX but management without an anti-metabolite was impossible and she is now on hydroxyurea.

In the light of our experience with Mr D.B. the MTX could probably have been continued and its use again in the future is not precluded.

Mrs E.C. Aged 69 years. She had an abnormal pre-MTX biopsy showing grade 1 fibrosis, but it was felt that the use of MTX could not be avoided. However, after 0·75 g this had progressed to grade 2 fibrosis with grade 1 inflammatory cell infiltrate, and the drug was discontinued.

Two patients (who do not appear in the prospective group of twenty five patients as they did not have pre-MTX liver biopsies) are mentioned as they only had weekly MTX therapy and both had very large cumulative doses.

Mr I.R. Aged 62 years. He had erythrodermic psoriasis, a history of schizophrenia and was on MTX by weekly intramuscular injection continuously for 11 years. If for any reason he discontinued his MTX he rapidly developed erythroderma and became psychotic and dangerously ill. After 10 years when the total cumulative dose of MTX was 13·3 g, his liver biopsy showed only mild changes—grade half inflammatory cell infiltrate and grade 1 fibrosis. There had been no progression since his first biopsy despite an intercurrent episode of chlorpromazine jaundice. He died in January, 1974, with carcinomatosis from a squamous cell carcinoma of the scrotum. He had had arsenic treatment for his psoriasis in the past. Permission for autopsy was not given.

Mrs E.A. Aged 65 years. She suffered from recurrent bouts of acute generalized pustular psoriasis and psoriatic arthritis mutilans. She was dangerously ill at the time the MTX was first started in 1964. Subsequently, she received a total of 10·6 g. She had had jaundice in the past and was suspected of heavy drinking. She always refused a liver biopsy, but by 1971 the liver function tests suggested cirrhosis and this diagnosis was finally confirmed at autopsy in February, 1974. She died of haemorrhage from a gastric erosion, jaundice, ascites and bronchopneumonia. Without MTX she would undoubtedly have died many years earlier from pustular psoriasis.

Liver biopsy findings in untreated severe psoriasis

Apart from the twenty-five patients analysed so far, forty-one other patients in need of MTX have had a pre-treatment biopsy only. The findings in this whole group of sixty-six patients (25+41) are shown in Table 3. These sixty-six patients form a sizeable sample of patients with psoriasis severe enough to warrant consideration of MTX and never previously exposed to this drug or any other of the antimetabolites used in psoriasis. One, an alcoholic, proved to have cirrhosis. Four others had fibrosis but only one of these had grade 2 changes. Similarly, although 17 out of 66 had some increase in inflammatory cells this only reached grade 2 in one patient.

TABLE 3. Liver biopsy findings in severe psoriasis in sixty-six patients

Normal	= 46	
Cells increased	= 17	only 1 patient grade 2
Fibrosis	= 4	only 1 patient grade 2
Cirrhosis	= 1	alcoholic

DISCUSSION

The abnormal liver biopsy findings in the pre-MTX group were minimal apart from the one cirrhotic who was an alcoholic. These findings extend and confirm our previous observations (Almeyda *et al.*, 1972) and do not support the concept of a psoriatic hepatopathy (Zachariae & Sogaard, 1973). Zachariae, Sogaard & Nyfors (1974) have shown that previous exposure to therapeutic arsenic does not influence the findings. The purpose of this on-going study has been to assess the hepatic risk of intermittent MTX therapy. It is fully recognized that the cumulative dosages are not very large in most of our patients, and it is not known what degree of liver damage may result with much higher cumulative doses. However, with the relatively small doses that were needed in these patients, usually about 0·7 g per year, it is likely that many years' treatment can safely be given to patients whose intractable psoriasis warrants it provided an intermittent dosage schedule is used. Equally encouraging data have recently been published elsewhere, demonstrating the safety of intermittent therapy (Reese *et al.*, 1974) but the data of Nyfors & Poulsen (1974) and Zachariae, Grunnet & Søgaard (1975) are less encouraging and in our view, the opinion of Reese *et al.* that liver biopsy is no longer needed with intermittent therapy is premature. Milward-Sadler & Ryan (1974) have again emphasized the increased hepatotoxic risk of daily as opposed to intermittent (usually weekly) schedules and the former should now have been abandoned.

CONCLUSIONS

(1) In this series of patients the prospective study has, as yet, shown little evidence of progressive hepatic structural damage from MTX given in weekly oral dosage.

(2) The results are in keeping with similar findings in a larger number of patients, also only treated by weekly dosage and subjected to several biopsies, but excluded here because pre-MTX liver biopsies were not performed (before 1969).

(3) This study has revealed fluctuations in the mild degrees of inflammation and fibrosis observed. It is not known whether these fluctuations are due to sampling error caused by focal pathological change, or are due to observer variation, or reflect truly fluctuating events.

(4) It is suggested that some of the hepatotoxicity reported earlier by this group and by others may be attributable to the now largely abandoned daily dose schedules and the failure to detect patients with abnormal liver histology before treatment.

ACKNOWLEDGMENTS

The authors are grateful to those colleagues who referred patients to us.

The London Hospital Research Fund and the Medical Research Council provided financial support.

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is responsible for the white banding in pellagra and in the patient with a carcinoid tumour, whose history is reported here.

Unfortunately we have no biochemical evidence in support of this suggestion because no preoperative estimations of serum 5-hydroxytryptamine or urinary 5-hydroxyindoleacetic acid were performed, as there was no clinical evidence of the carcinoid sydrome.

ACKNOWLEDGMENTS

We should like to thank Professor Gowing of the Royal Marsden Hospital Pathology Department and Dr Hinson of the Pathology Department of the Brompton Hospital for their reports on the histology of the skin lesion and bronchial tumour respectively, Dr Citron and Mr Lennox of the Brompton Hospital and Dr G. Hodgkinson of King Edward Memorial Hospital for their help in the clinical management of this patient, and the photographic department of the Royal Marsden Hospital for the photographs reproduced in this paper.

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British Journal of Dermatology (1975) 93, 347.

Brief Communications

Sebum excretion rate investigations

A NEW ABSORBENT PAPER

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One of the problems of assessing sebum excretion rate (SER) by the gravimetric method of Strauss & Pochi (1961) is the need for a suitable absorbent paper. Cunliffe & Shuster in 1969 made it quite clear that many papers, even though reasonable in appearance, vary tremendously in their ability to absorb surface lipids. The original paper which has been used by the Newcastle Group, the Leeds Group and more recently by the Bristol Group was obtained from Brittons Limited in Leek. However, that particular paper is now no longer available. Over the past 6 months we have investigated other papers and compared them to the original obtained from Brittons. We have now found a paper which is as good as the original one and we would recommend it for the use of dermatologists and of basic scientists who are interested in measuring SER. Table 1 shows the SER in eight people; the sebum being collected from the right and left sides of the forehead with the old and new papers respectively. A paired Student '*t*' test demonstrated that there was no significant difference ($P<0\cdot9$). The paper (special velin tissue, non-fluff) is obtainable from: Koch-Light Laboratories Ltd., Willow Rd., Poyle, Colnbrook, Bucks.

TABLE 1. Values of SER in $\mu g/cm^2/min$ obtained with old and new papers in eight people

Old	New
1·85	1·94
1·54	1·01
1·06	1·73
0·21	0·24
1·28	1·18
1·26	1·03
0·58	0·62
0·67	0·62

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Electron microscopy of the sweat glands from the hairy skin of the beagle

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Accepted for publication 14 March 1975

Recently Bell & Montagna (1972) published the results of a light histochemical study of sweat glands in the hairy skin of horses and dogs. They found that there was no demonstrable innervation of these glands in the dog. We have previously reported stimulation of the sweat glands on the hairy surface of the beagle by hypothalamic (Cotton, van Hasselt & Bergers, 1975) and peripheral nerve stimulation (Cotton & van Hasselt, 1972) but the stimulus levels were so high that we believe the effects to be unphysiological and secondary. The only effective physiological stimulus that we have found is copulation (Cotton *et al.*, 1975). Other authors have found that the kangaroo sweats during physical stress in a treadmill but not in response to heat (Dawson, Robertshaw & Taylor, 1974).

The data in the literature indicate that the sweat glands on the hairy surface of the dog are apocrine (Iwabuchi, 1967) and it is well known that the development of human apocrine glands is associated with sexual maturity (Zelickson, 1967). On the basis of these observations we have tentatively suggested (Cotton *et al.*, 1975) that these glands may have a pheromone (Mykytowycz & Goodrich, 1974) function in the dog.

Since the structure of these glands has only been studied at the light histological level, we thought that it might be of interest to examine them under the electron microscope. We have sectioned a number of sweat glands obtained from the hairy skin of the dog and the following morphological details are apparent. In no section did we observe any nerve ending although these were readily discernable in human eccrine and dog foot pad preparations. Only two types of cells were visible, myoepithelial cells and secretory cells, containing many inclusions and bordered with microvilli (Cotton *et al.*, 1975). The secretory cells contained the atypical mitochondria with amorphous matrix as described by Zelickson (1967) and sweat glands obtained from areas which had been stimulated to sweat by local injections of acetylcholine showed extended apical regions free of microvilli and inclusions.

Consequently we conclude that these glands are apocrine and lack direct innervation. These conclusions, although not providing direct proof, are in accordance with the idea that the glands may serve a social communication function in addition to any as yet unclear, thermal regulation function.

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Hair fragment in the skin resembling larva migrans

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Accepted for publication 7 February 1975

Hair fragments penetrating the skin may produce a variety of different clinical pictures (Scott, 1957). One of the most peculiar of these resembles larva migrans (Yaffee, 1957; Schamberg, Park & Strauss, 1961; Ronchese, 1962).

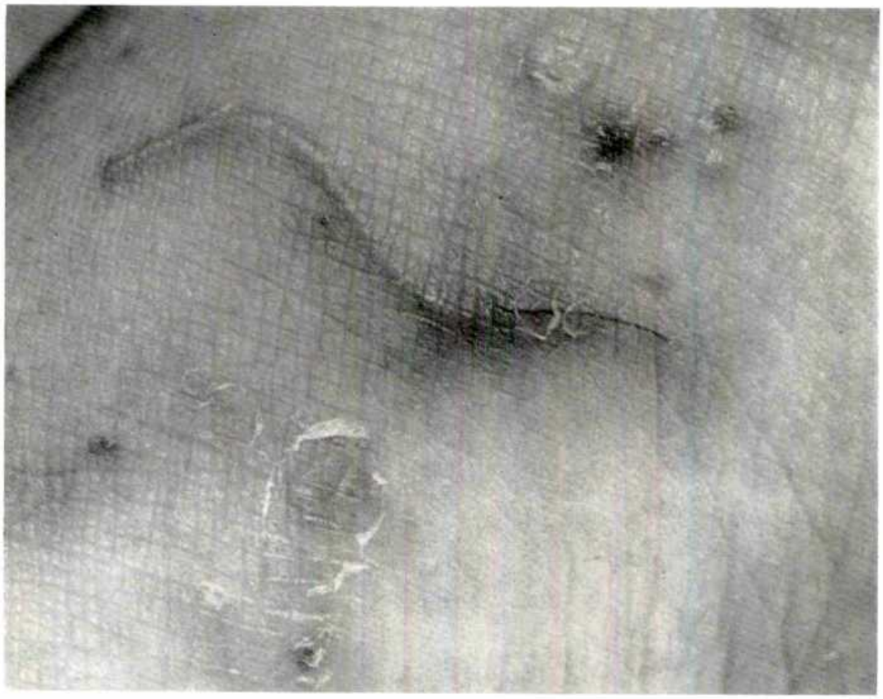

FIGURE 1. Hair fragment on medial side of the metatarsal area of the right foot.

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A 2-year-old boy was admitted for the treatment of scabies. On examining his feet we found to our surprise a 35 mm long wavy superficial burrow on the medial side of the metatarsal area of the right foot. In its lower end there was a thin dark structure 9 mm long (Fig. 1).

Our initial diagnosis was larva migrans, but all cases diagnosed in Finland have so far been acquired in tropical areas, and this patient had never been abroad. During a few hours of inspection it was noticed that the 'larva' had moved about 7 mm backwards in its burrow. On opening the burrow the 'larva' turned out to be a dark hair fragment. It differed from the scalp and beard hair of his father. It was medullated and closely resembled a dog's hair, but the family had no domestic animals. It was not as coarse as a bristle.

In all reported cases such a hair fragment has been found in feet and the friction between shoes and feet seems to be a predisposing factor.

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Comment

THE SAFETY OF FRAGRANCE INGREDIENTS

Fragrance raw materials are derived from many geographical, natural and chemical sources. Any programme of safety evaluation has to consider the materials' origins, types of uses and the concentrations used.

An institute, the Research Institute for Fragrance Materials, Inc., an international non-profit organization, has been established by the industry to do research on nearly 5000 ingredients employed in perfumery. This institute (RIFM) was established in 1966 by the principal suppliers of raw materials to the fragrance industry. At present, RIFM is supported by forty-five of these companies, representing nearly 97% of the industry in the United States, Europe and Japan. The sole purpose of RIFM is to assure the safety of perfumery raw materials.

Raw materials are selected on the basis of the following criteria: (1) they must be representative of the material *in actual use* by the industry; (2) they must conform to the specifications and standards, if any, of the Essential Oil Association of the USA(EOA) or of the International Fragrance Association (IFRA); (3) they must be supplied to the RIFM without indication of the supplier, with name and an identification number only; (4) they must be accompanied by gas-chromatographic, ultra-violet or infra-red curves to 'thumb-print' the materials.

The specifications and standards in the monographs will be those of the EOA, where available. The levels of usage reported are the results of industry-wide surveys.

The chief parameters selected for toxicological screening of all these materials include tests for allergenicity and phototoxicity on human skin; tests for general toxicity in animals by the oral and dermal route, and other procedures where required by an International Panel of Experts drawn from outside the industry.

Several allergens and phototoxic materials have been reported. The ubiquity of fragrances in cosmetics, household goods, insect repellents, etc., makes the responsibility of such an institute a significant one, and it is hoped that this ingredient approach, responded to zealously by the industry, will eliminate potential offenders from fragrances world wide.

RIFM has now completed the evaluation of 530 of the fragrance raw materials in greatest use, representing about 90% of the volume of fragrances. The programme is continuing on the smaller items. Of these 212 have been published in *Food and Cosmetics Toxicology* (1973, **11**, 95–115, 477–495, 855–876, 1011–1081; 1974, **12**, 385–405, 517–537, 703–736, 807–1016; 1975 **13**, 91–112).

Included in the monographs are average and maximum levels of usage of the materials in soaps, detergents, creams and lotions and perfumes; regulatory status; measurements of acute toxicity, irritation, allergenicity and phototoxicity; as well as citations of pertinent literature.

These monographs are available without charge to interested parties either from: International Fragrance Association, 8 Rue Charles-Humbert, 1205 Geneva, Switzerland, or from: Research Institute for Fragrance Materials, Inc., P.O. Box 1152, Englewood Cliffs, New Jersey 07632, U.S.A.

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British Journal of Dermatology (1975) 93, 353.

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ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN LICHEN PLANUS

SIR, Cotton *et al.* (1972) studied glucose-6-phosphate dehydrogenase (G-6PD) activity in biopsies of lesions from patients with lichen planus and suggested that this skin condition might be associated with a congenital abnormality of the enzyme in the skin. It has been shown that in subjects whose erythrocytes have deficient G-6PD activity, cultured skin cells are also deficient (Gartler *et al.*, 1962; Davidson *et al.*, 1963). The increased incidence of lichen planus in the tropics (Shrank & Harman, 1966) coincides with the prevalence of erythrocyte G-6PD deficiency in the same geographic areas. There appear to be no publications on the incidence of lichen planus in patients with erythrocyte G-6PD deficiency or on the frequency of this enzyme abnormality among lichen planus patients.

The intent of this study was to record the incidence of erythrocyte G-6PD deficiency in a group of patients with lichen planus. Haemoglobin electrophoretic pattern was simultaneously determined following the observation of Smits *et al.* (1969) that sickle cell crises may be more frequent in individuals with concomitant G-6PD deficiency.

TABLE 1. The composition of G-6PD activity and Hb electrophoretic pattern in patients with lichen planus and in control group

	G-6PD activity			Hb electrophoretic pattern		
Group	Normal activity	Partial deficiency	Complete deficiency	AA	AS	AC
Lichen planus	24	3	1*	23	6	1
Controls	21	5	4	24	6	0

* Female, the remaining subjects with partial or complete G-6PD deficiency were males.

Erythrocyte G-6PD activity was estimated by the semiquantitative method of Motulsky using SIGMA kit (Berger, 1971). Estimations have been performed in thirty Nigerian patients (23 males and 7 females) with lichen planus and thirty controls. Control patients were matched for age and sex and included persons suffering from scabies and acute skin pyogenic infections. All patients showed typical lesions of lichen planus and in twenty-two cases the diagnosis had been confirmed histologically. The pattern of haemoglobin was estimated electrophoretically.

There was no difference in incidence of erythrocyte G-6PD deficiency and haemoglobin patterns between lichen planus and control subjects (Table 1).

This study demonstrates that there was no difference in the activity of erythrocyte G-6PD between a group of patients with lichen planus and a control group in an area with a frequent incidence of this enzyme deficiency. Similarly, no cases of lichen planus have been observed in a large series of G-6PD deficient patients seen in Zaria and Kaduna Hematologic Clinics (Fleming, 1975). Obviously the results only show that the activity of the enzyme is within normal limits in erythrocytes of patients with lichen planus and this does not disagree with the concept of Cotton *et al.* (1972) suggesting G-6PD deficiency in the skin. This is a preliminary investigation using a relatively insensitive method and a study of quantitative estimation of G-6PD in red cells of lichen planus patients is now in course.

ACKNOWLEDGMENTS

Thanks are due to Professors A.F.Fleming and E.H.C.Parry (Ahmadu Bello University Zaria) for their advice and encouragement. The skilled technical assistance of Mr S.D.Jikeme and Mr Y.A.Ogunfunmilade is gratefully acknowledged.

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A reply

SIR, the observations reported by Dr Jacyk are very interesting and concern a problem that we have been wondering about for some time. Most of the data in the literature indicate that the G-6PD in the skin is the same as that in the erythrocytes since, among other things, skin biopsies can be used to diagnose favism (Gartler *et al.*, 1962). However, it is also known that there are various isozymes of G-6PD in the skin (Davidson *et al.*, 1963) and the possibility remains that one or more of these isozymes may be quantitatively or even qualitatively peculiar to the skin and be coded for independently of the erythrocyte isozyme responsible for favism. We have examined a few electrophoretic patterns of G-6PD in the skin of lichen planus patients but could find no differences between these and similar extracts from the skin of normal subjects (Mier & van den Hurk, unpublished observations). But there is, of course, no *a priori* reason why a functional enzyme abnormality should always be reflected in a changed electrophoretic mobility.

There are two specific objections to our previous work (Cotton *et al.*, 1972) which mature reflection has made apparent. The first is that we took full depth punch biopsies which, in the case of lichen planus lesions, would include the characteristic band-like infiltrate. Thus if the infiltrate cells contained an unusual G-6PD isozyme this could result in an apparent K_M change. The second objection is that some of our patients had been taking oral steroids and we now find that some steroids can inhibit G-6PD. According to our calculations the quantity of steroids in the patients was too low to produce a significant inhibition but since accumulation of steroids in the skin cannot be ruled out such an effect must remain a possibility.

As Dr Jacyk remarks, the test that he used was only semiquantitative and presumably measured some function of the V_{MAX}. It is well established that many G-6PD variants have a normal V_{MAX} but an altered K_M (Harris, 1970). Since we reported a normal V_{MAX} and an altered G-6PD K_M we would not expect a semiquantitative test to reveal this difference.

Nevertheless it is very interesting to see that there is no overlap between favism and lichen planus biochemically, in spite of what might be expected on the basis of theory and the geographical distribution of both diseases.

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Book Reviews

The Structure and Function of Skin. WILLIAM MONTAGNA and PAUL F.PARAKKAL (1974) 3rd edn. London: Academic Press. Pp. 433, 162 figures. Price £12.95.

I do not think it is too much of an exaggeration to suggest that if embryonic dermatologists were to read this and no other book during gestation, there still would be an overall improvement in the level of understanding of the skin as a functioning organ. If these dermatological fetuses were also to consult the 52 odd pages containing references and then actually read some of the references therein, I predict that at term we would have a group of very well educated dermatological neonates indeed. This book is a concise and scholarly work. It neatly summarizes present knowledge concerning the various cutaneous nuts and bolts, the way these fit together and the way that cutaneous structures work. It is extremely well illustrated and some of its figures grab the imagination and stimulate the wondering gland. For example, some of the remarkable photomicrographs of histochemical preparations of the innervation and blood supply to the pilosebaceous follicle excite many questions concerning the growth interrelationships between the mesodermal and ectodermal components of this very complex structure.

However, although the figures promote questions the text rarely does. The information perhaps underlines too sharply what is known and not what we need to know. I suppose the requirements of brevity dictated that the authors could not devote a great deal of space to speculation on for example, the function of Langerhans cells, or on the control mechanisms for epidermopoiesis. This criticism aside, I think that it is an excellent book. It is one that should be read by all dermatologists in training as well as by all others who retain a lively interest in the nature of the organ whose diseases they treat.

R.MARKS

Sunlight and Man. Normal and abnormal photobiologic responses. T.B.FITZPATRICK (Consulting Ed.), M.PATHAK, L.HABER, M.SEIJI & A.KUKITA (Eds.) (1974) Tokyo: University of Tokyo Press. Pp. 870. Price £31.80.

The clinician is concerned with trying to classify forms of reaction of the skin to ultraviolet radiation (UVR) and to visible light so that common ground will exist for the discussion of what is known of these various 'photodermatoses'. At the same time, he tries to assess the importance of photoactive substances whether derived from exogenous or endogenous sources, and to understand whether immunological processes are involved or whether it is just a matter of too much substance and/or too much radiation. He wants to know how to confirm 'photosensitivity' and to define, where possible, the responsible wavelengths and what equipment he requires for this and what he needs to do to ensure its reliability. This information is necessary to allow him to diagnose, advise, and treat the patient. The experimental photobiologist is also concerned with these problems, but is at the same time involved in the study of the fundamental changes occurring in normal skin following exposure to UVR and visible light and in the skin with added photoactive substances or repair mechanism defects, etc.

'Sunlight and Man' is the outcome of an international conference held in Tokyo in 1972 and is based on a series of symposia chosen to reflect progress in the field of photobiology in the preceding 20 years. This book is divided into sections covering (1) the action of light on biological systems, which deals with the fundamental changes in the skin resulting from exposure to light and the mechanisms of repair: (2) the action of light on man and mammals, which discusses the effects of solar irradiation of the skin, the production of erythema by both short and long UVR, and the biochemical evidence for the effect on the lysosomes and cell membranes; the relationship of melanin pigmentation to solar irradiation damage; the biological consequence of living under 'lighting environments' which differ considerably from the environment in which man evolved, and a plea for the greater use of phototherapy in medicine; carcinogenesis and the importance of environmental chemicals in association with UVR: (3) the action of light on the skin and the presence of exogenous photosensitizing agents such as psoralens, dyes, chlorpromazine, tetracycline, sulphonamides, with a discussion of the evidence for phototoxicity and photoallergy and a consideration of the light sources used for photopatch testing and the determination of action spectra: (4) the action of light in the presence of endogenously produced photosensitizing metabolites and the metabolic, histochemical, clinical, and therapeutic aspects of the porphyrias: (5) the clinical and photobiological aspects of the idiopathic photodermatoses (polymorphic light eruption, actinic reticuloid, etc.): (6) phototherapy and photoprotection in which normal protective mechanisms and the choice and efficacy of currently available light-protecting substances are discussed, and also the use of phototherapy in the treatment of vitiligo and psoriasis; and lastly: (7) the technique of patch testing and the terminology and physical units of radiation commonly used in photobiology.

The organizers and editors have succeeded in their purpose of collecting into one volume an assessment of most of the important aspects of cutaneous photobiology, and in their choice of the clinicians and research workers suited to the task. It would be surprising if all the views expressed were acceptable to an individual reviewer but the lack of agreement on the clinical and photobiological aspects of the photodermatoses and a perpetuation of some of the confusion which exists in the mind of the practising dermatologist is regretted. It may be that this confusion will last as long as our ignorance of the causation of these common clinical syndromes.

All concerned with this production are to be congratulated. It is an essential work of reference for hospital Departments of Dermatology.

W.FRAIN-BELL

Atlas de Mycologie Médicale. JEAN DELACRÉTAZ, DODÉ GRIGORIU and GEORGES DUCAL (1974)
Paris: Masson et Cie. Pp. 180. Price £47.70.

The standard of production of this book is very high and the generally excellent clinical photographs, photographs of fungal cultures, photomicrographs of fungal preparations and histological sections are in colour. Also included are illustrations of two electronmicrographs and several X-rays.

It would be helpful if all illustrations were numbered, instead of only some of them. It would also be useful if the magnification of microscopic preparations was given and more reverse sides of cultures shown. The blue background tends to distort the colour of some cultures.

The text, as befits an atlas, is succint. The opening chapters deal with the biology and classification of fungi and the collection and examination of specimens. Brief mention is also made of intradermal tests, biopsies and serological tests. The inexperienced mycologist will be pleased to see good photographs of artefacts.

A more detailed chapter on dermatophytes is followed by chapters covering the different aspects of dermatophyte infections, yeast infections and the deep and visceral mycoses. Candida granuloma is separated from muco-cutaneous yeast infections by 8 chapters. Erythrasma and actinomycosis are included, the former, at least, for traditional reasons. The clinical nomenclature may seem unusual to British dermatologists but the illustrations make all clear.

The authors have been purposely brief when dealing with treatment. On the whole, treatment suggested is conventional but mercury compounds are suggested for topical use and radiotherapy for kerion and chronic paronychia.

The dermatologist doing his own mycology will be interested in the chapter on contaminants and in the illustrated glossary at the end of the book.

In their introduction the authors write that this book is intended for all doctors whether dermatologists or not. They will certainly enjoy it and the atlas will be of particular interest to those amateur mycologists among them who will probably wish to possess it. It would be a useful addition to Dermatology Departments and General Medical Libraries.

MARGARET M.WALSHE

Recent Advances in Sexually Transmitted Diseases. R.S.MORTON and J.R.W.HARRIS (Eds.) (1975) Edinburgh: Churchill Livingstone. Pp. 420. Price £10.00.

This book is a worthy successor to the Recent Advances in Venereology of 1964. The change in title to 'Sexually Transmitted Diseases' is necessitated by the inclusion of many diseases not legally defined as venereal but often sexually transmitted. The nine expert contributors have culled the literature from 1964 to 1973 and have presented a balanced summary.

The book is divided into nine parts; the first deals with gonorrhoea. The various opinions for the increased incidence are put forward. More frequent reports of complications such as salpingitis, septicaemia with arthritis and the 'gonococcal dermatitis syndrome' represent a failure of contact tracing. Ignorance about the asymptomatic carrier, both male and female, contributes to the failure of control. The GCFT is still not an effective diagnostic aid. Treatment schedules must relate to the *in vitro* sensitivity of the *Gonococcus* and their efficacy be checked with subsequent repeated cultures, not only from the usual sites, but also from pharynx and rectum. A vaccine is a long way off.

The second part deals with syphilis which is better controlled in this country than in many others. Early syphilis is commoner amongst male homosexuals than heterosexuals. The persistence of treponeme-like forms after penicillin for late syphilis has aroused much debate about their meaning. Advances have been made in serological tests such as the treponema pallidum haemagglutination test (TPHA), though the treponema pallidum immobilization test (TPI) is still the final arbiter. The chapters on syphilis serology and the biological false positive (BFP) reaction with its relationship to auto-immune diseases are valuable to all physicians.

Parts 4 and 5 deal with trichomoniasis and candidiasis; their frequent association with genital warts is noted.

Part 6 on non-specific urethritis (NSU) with special reference to the chapter on laboratory aspects is an excellent review of this common and troublesome disease. Many venereologists would question the statement that no such disease as NSU occurs in women.

Part 8 is a useful section which includes herpes genitalis, molluscum contagiosum, genital warts, pediculosis pubis and scabies.

The final part deals with control of infection by provision of an adequate network of clinics, health education and thorough contact tracing.

This book should be in the library of all venereologists and in the postgraduate centres for reference by those in many other disciplines.

J.B.LYON
P.M.WRAY

Writing Scientific Papers in English. An ELSE-CIBA Foundation Guide for Authors. MAEVE O'CONNOR and F.PETER WOODFORD (1974) Amsterdam: Elsevier–Excerpta Medica–North Holland. Pp. 108. Price £3.50.

This little book was produced by ELSE (European Association of Editors of Biological Periodicals) and by the Ciba Foundation as an introductory guide for authors of any nationality submitting papers to English language scientific—not necessarily medical—journals.

Much of the advice it contains might be taken for granted, but advice that is all too often not heeded. It is an account by two very experienced authors of how to set about putting one's thoughts in order, and then how to put pen to paper, and even how to prepare the manuscript and to deal with editors and with proofs. The advice ranges from such general remarks as 'do not try to spin several short papers out of what is essentially one investigation', to more precise details of how to cope with illustrations, copyright, etc. It also includes some advice on the difficulties of the English language, but does not pretend to replace, nor is it so entertaining as, Gowers' 'Complete Plain Words' or Fowler's 'Modern English Usage'.

Even experienced authors could enjoyably and profitably spend an hour reading through this book, although it is meant mainly for those less experienced authors. Many editors will hope that it sells well.

R.H.CHAMPION

Immunological Aspects of Allergy and Allergic Disease. Edited by E. RAJKA and S. KOROSSY (1974) London: Plenum. Volume 1. Basic Concepts in Experimental Immunology. Pp. 456. Volume 2. Methods in Experimental Immunology. Pp. 292.

This massive work has grown out of a monograph first published in 1959 in German and later in Russian. With this revised and up to date edition the opportunity has been taken to produce an English version.

The two volumes present an ambitious review of theoretical and experimental immunology and two further volumes dealing with clinical aspects are promised. There are twenty-seven contributors, all but five of whom are from Hungary.

The first volume 'Basic Concepts in Experimental Immunology' is divided into two sections. The first deals with the fundamentals—definitions, nomenclature, classification and pathogenesis of immune responses and an account of the Shwartzmann phenomenon is included. The second section covers, more fully, antigens, immunoglobulins and antibodies, cellular aspects of immunity, the thymus and the various mediators of inflammation. The final chapters deal with immunosuppression and anti-lymphocyte serum.

Volume 2 'Methods in Experimental Immunology' is not as comprehensive as its title implies, since the practical details of the experimental methods are all too often not given and this book cannot be recommended as a handbook for the laboratory shelf. Patch testing is dismissed in a single paragraph but more space is devoted to mucosal testing—nasal, bronchial and even urethral and rectal. The hazards of intracutaneous skin tests are vividly described as are the necropsy findings. There is, however, no word on the management of the events preceding the necropsy.

These tomes provide a collection of reviews of current thinking and the state of knowledge about allergy and immunology. Numerous references are given for each chapter and the European literature is especially well covered, so that the work is a useful source of references. However, many of the contributions suffer from the fact that English is not the native language of their authors.

J.J.CREAM

Viruskrankheiten der Haut der Schleimhaute und des Genitales. THEODORE NASEMANN, in collaboration with GERTRUD SCHAEG and OTTO SCHULTA (1974) Stuttgart, Georg Thieme Verlag. Pp. xii + 284, 232 illustrations, 12 colour plates, 32 tables.

Dr Nasemann, Professor of Dermatology at Frankfurt am Main, is well known for his many publications on virus infections. His new book, which is intended for the practising doctor, presents an admirably clear and up-to-date account of virus infection of the skin and mucous membranes, and of the genitalia. The numerous illustrations are of such unusually high quality that it is appropriate that the name of the photographer, Otto Schultka, should appear on the cover of the book, together with that of Mrs Schaeg, the author's technical assistant.

The first part of the text is a very readable introduction to general virology, designed for the non-virologist. There follows a systematic account of the individual viruses, and of the pathology, clinical features and management of the diseases with which they are associated. The twelve full-page colour plates are s good that, supplementing as they do the numerous black and white illustrations, they should provide real help with some diagnostic difficulties. The treatment of each disease is briefly but adequately summarized, but proprietary preparations are too often referred to only by their German trade names, which differ from those in use in most other countries. It would have been helpful too to have been provided with the evidence from controlled trials, of the efficacy of some of the procedures recommended.

Some authorities are cited in the text but no references are provided; the book ends with a short bibliography which lists a selection of standard works in English and German, and a few articles in journals.

The book is well produced and attractively designed and succeeds admirably in fulfilling the author's objective of providing a manual for the dermatologist or general practitioner. The effective layout, the illustrations and the tables should make the book useful in the clinic even for those whose ability to read German is rudimentary.

Arthur Rook

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
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British Journal of Dermatology, Volume 93, Number 3, September 1975

CONTENTS

Clinical and Laboratory Investigations

Page

Intra-epidermal Carcinoma of the Eyelid Margin 239
D. I. McCALLUM, P. D. C. KINMONT, D. WYNN WILLIAMS, R. E. COTTON AND M. A. WROUGHTON

Is the Cylic AMP in Psoriatic Epidermis Low? 253
K. YOSHIKAWA, K. ADACHI, K. M. HALPRIN AND V. LEVINE

Survey of Skin Disorders in the Urban Black Population of South Africa 259
M. DOGLIOTTI

A Method of Liberating Living Cells from the Dermal Infiltrate: Studies on Skin Reticuloses and Lichen Planus 271
R. S-H. TAN, N. A. BYROM AND J. P. HAYES

Thin Layer Chromatography and Counter-current Analysis in Porphyrias 277
J. PIÑOL AGUADÉ, C. HERRERO, J. ALMEIDA, S. G. SMITH AND R. V. BELCHER

The Use of Thin Layer Chromatography in the Separation of Free Porphyrins and Porphyrin Methyl Esters 291
S. G. SMITH

Further Studies of Scratching During Sleep 297
J. A. SAVIN, W. D. PATERSON, I. OSWALD AND KIRSTINE ADAM

A New Method for the Measurement of Itch and the Response to Treatment 303
R. FELIX AND SAM SHUSTER

✓Coexistence of Lichen Planus and Bullous Pemphigoid: an Immunopathological Study 313
G. STINGL AND K. HOLUBAR

A Prospective Study of the Effects of Weekly Oral Methotrexate on Liver Biopsy: Findings in Severe Psoriasis 321
A. P. WARIN, J. W. LANDELLS, G. M. LEVENE AND H. BAKER

Case Reports

Cowden Disease: Report of Two Additional Cases 329
JOSEPH W. BURNETT, RONALD GOLDNER AND GARY J. CALTON

Deafness Complicating Mucous Membrane Pemphigoid: a Case Report 337
JOHN THOMSON, WILLIAM LANG AND J. A. CRAIG

Unusual Cutaneous Associations of a Malignant Carcinoid Tumour of the Bronchus—Erythema Annulare Centrifugum and White Banding of the Toe Nails 341
J. D. EVERALL, P. M. DOWD AND B. ARDALAN

Brief Communications

Sebum Excretion Rate Investigations: a New Absorbent Paper 347
W. J. CUNLIFFE, S. M. WILLIAMS AND S. G. TAN

Electron Microscopy of the Sweat Glands from the Hairy Skin of the Beagle 348
D. W. K. COTTON, P. VAN HASSELT AND A. M. S. BERGERS

Hair Fragment in the Skin resembling Larva Migrans 349
EERO A. LEHMUSKALLIO

Comment

The Safety of Fragrance Ingredients 351
DONALD L. OPDYKE

Correspondence

Erythrocyte Glucose-6-phosphate Dehydrogenase in Lichen Planus 353
W. K. JACYK, D. W. K. COTTON AND W. B. J. M. VAN DER STAAK

Book Reviews 355

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Volume 93, Number 4, October 1975

British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

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1. Brit. J. Derm. **92**, 585, 1975
2. Archs. Derm. **86**, 608, 1962
3. Brit. J. Derm. **90**, 197, 1974

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Outline of Contents

Dermatoses due to fungi
Dermatoses due to viruses
Treponematoses
Dermatoses due to bacteria
Dermatoses due to animals
Dermatoses due to malnutrition
Exogenous and endogenous eczemas, lichen simplex and prurigo
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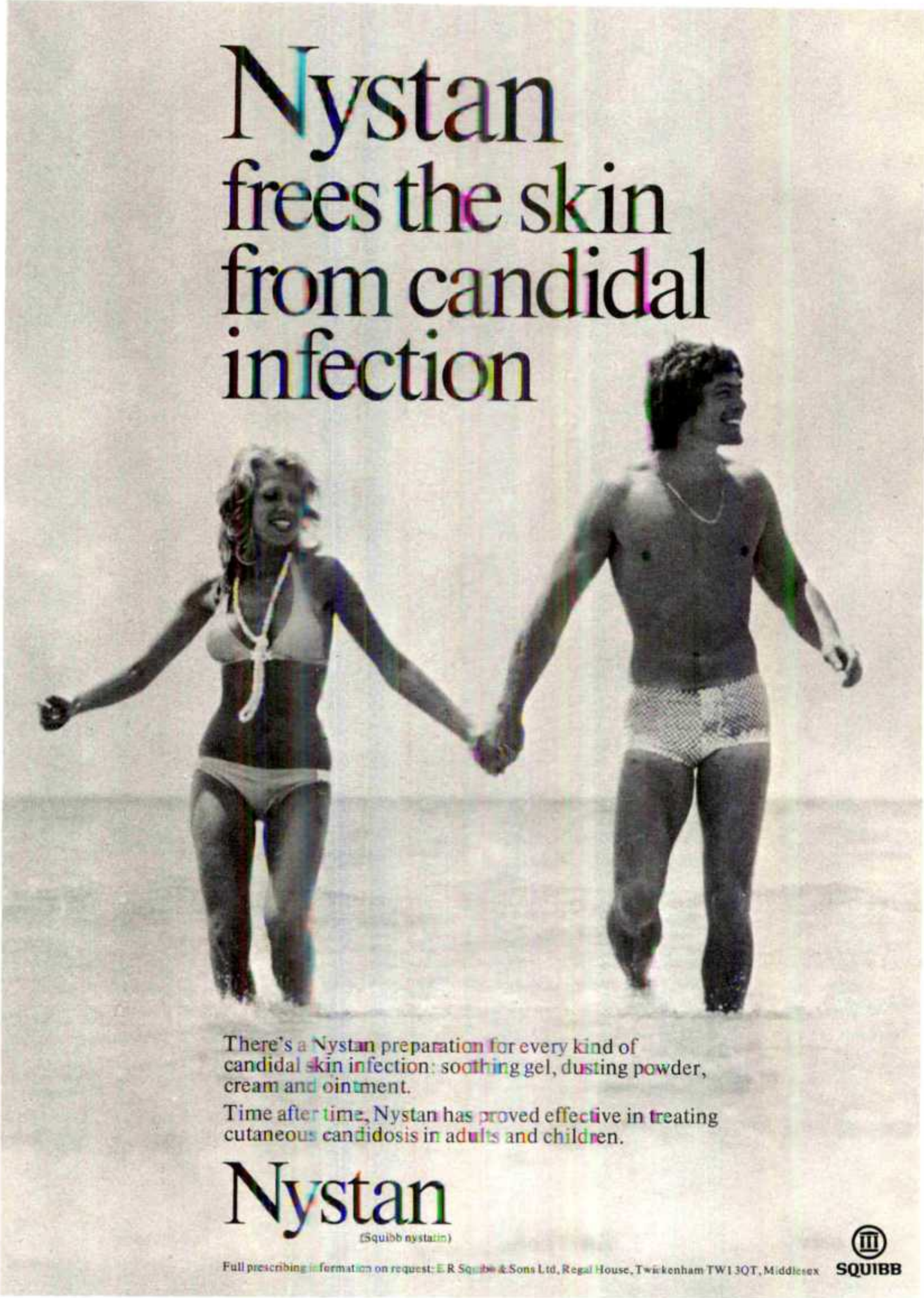

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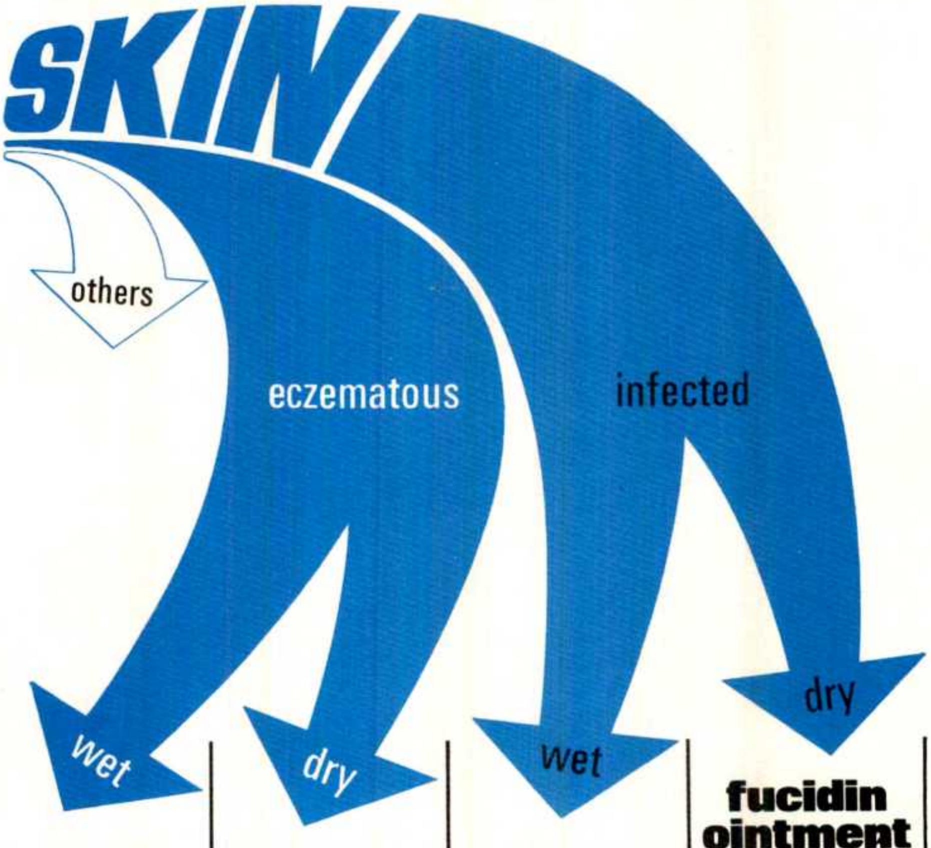

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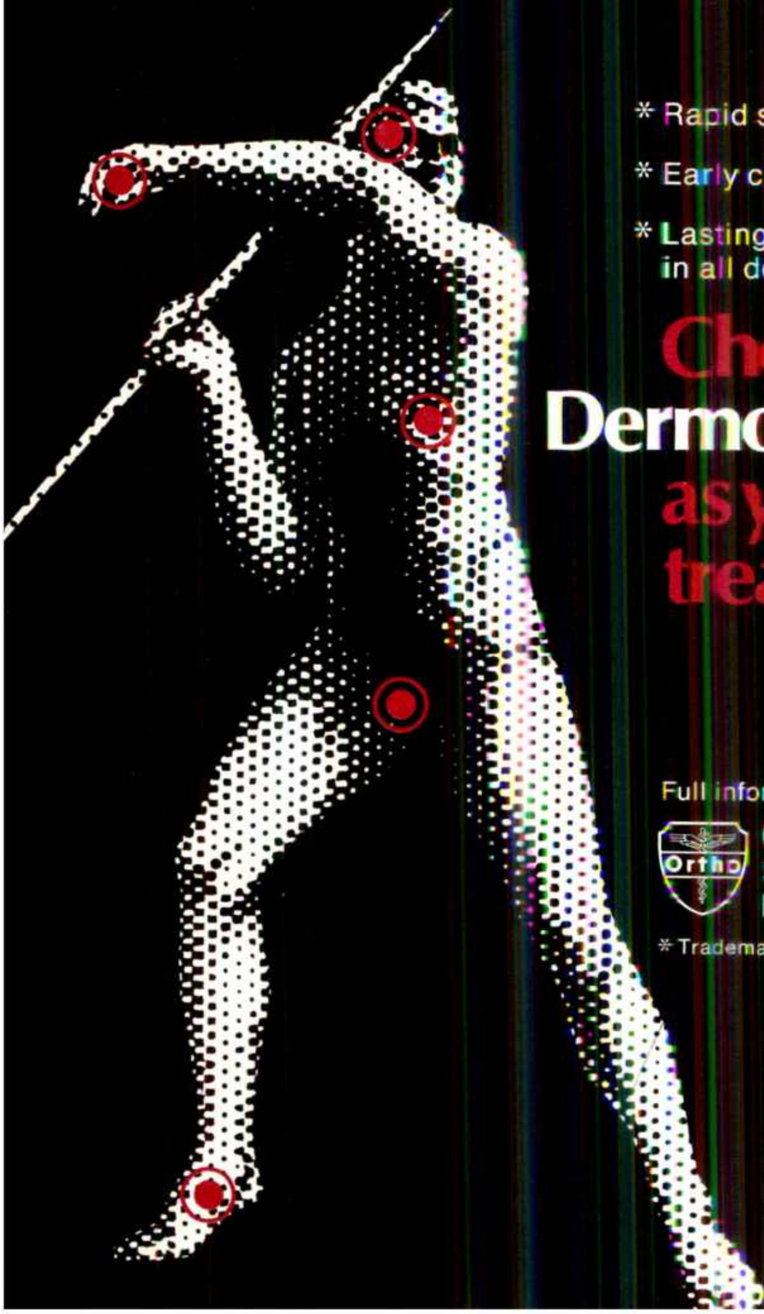

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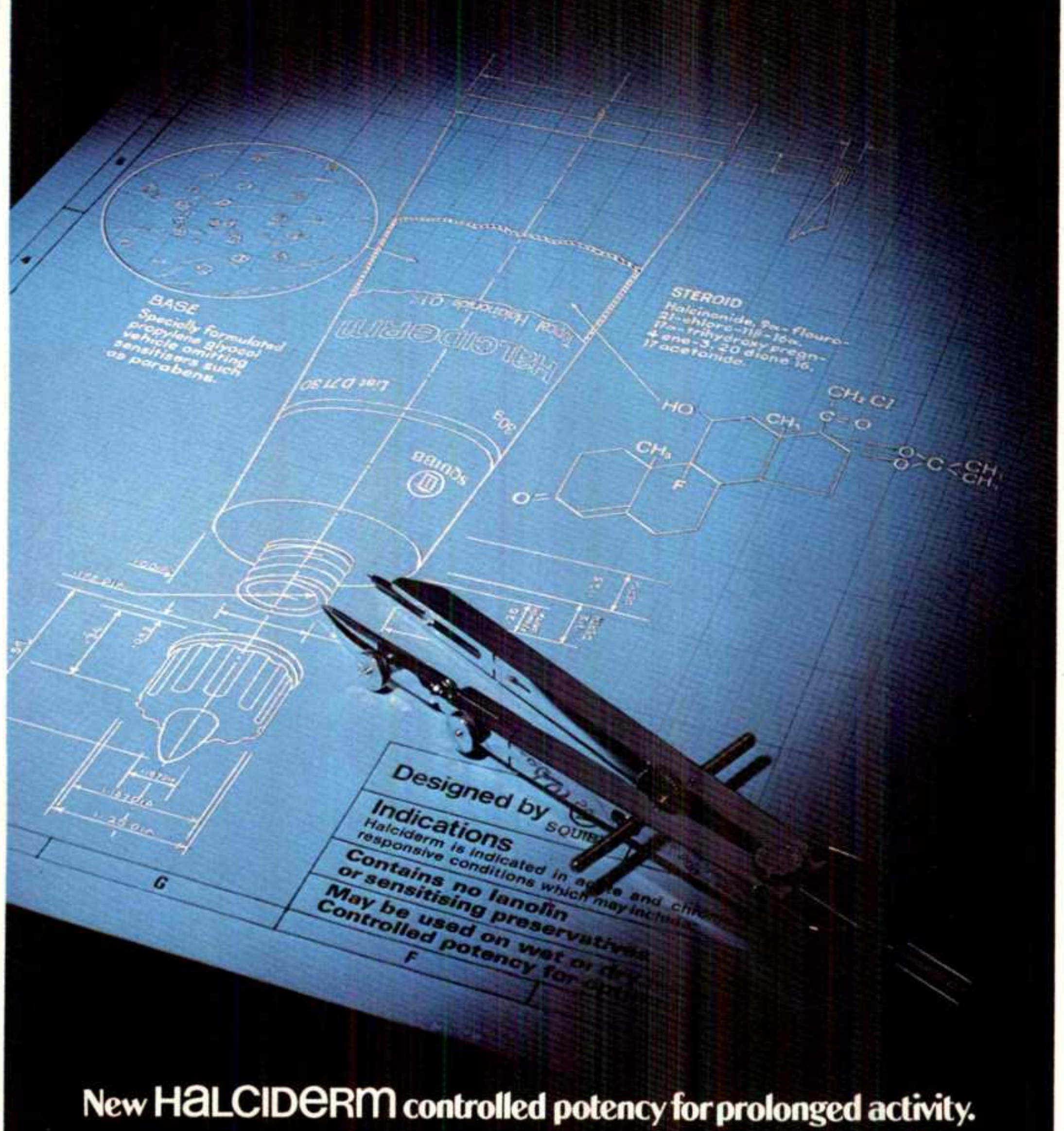
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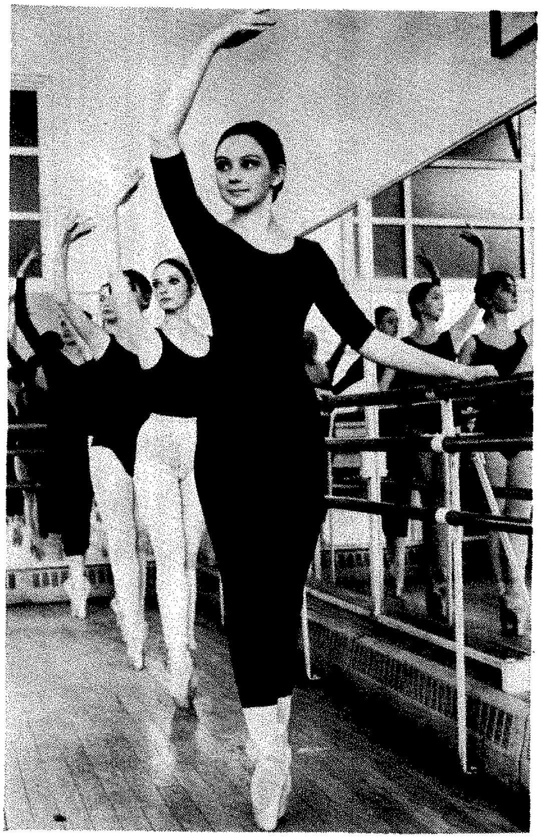

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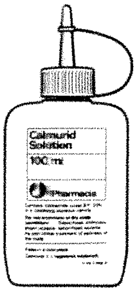

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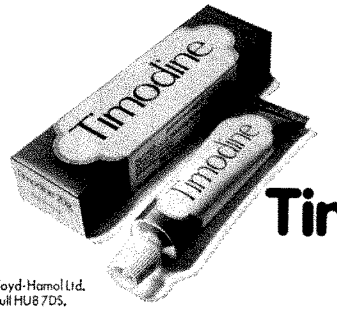

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Index Vol. 150, No. 3, 1975

Editorial

Schnyder, U.W. (Heidelberg): Genetics and Dermatology

Baker, H. (London): Psoriasis: A Review. II

Diezel, W.; Meffert, H. und Sönnichsen, N. (Berlin): Untersuchungen zum Wirkungsmechanismus von Dithranol: Erhöhte Lipidperoxydation und Enzymhemmung (Experiments Concerning the Mode of Action of Dithranol: Increased Lipid Peroxidation and Enzyme Inhibition)

Haim, S. (Haifa): Behçet's Disease: Etiology and Treatment

Rao, B.N.; Satyanarayana, B.V.; Venkatarathnam, G., and Reddy, C.R.R.M. (Visakhapatnam): 'Isopathic Phenomenon' in Leprosy

Zala, L.; Ettlin, Ch. und Krebs, A. (Bern): Fokale dermale Hypoplasie mit Keratokonus, Ösophaguspapillomen und Hidrokystomen
(Focal Dermal Hypoplasia with Keratokonus, Papillomatosis of Esophagus and Hidrocystomas)

Driban, N.E. and Lacognata, J.J. (Mendoza): Subungual Squamous Cell Carcinoma

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Clinical and Laboratory Investigations

Pigmented basal cell epithelioma

LIGHT AND ELECTRON MICROSCOPIC STUDIES ON TUMOURS AND CELL CULTURES

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Accepted for publication 10 January 1975

SUMMARY

Light and electron microscopic studies have been carried out on three pigmented basal cell epitheliomas and on cell cultures of two of these tumours, to determine the formation and transfer of pigment and the fine structure of the constituent cells. Scattered in the epithelial tumour buds were many functional melanocytes that contained melanosomes in various stages of development and whose morphology was essentially normal. Many melanophages were present in the dermal stroma, but few transferred melanosomes were to be found in the epithelial tumour cells.

Tissue culture studies revealed that melanocytes were present among the proliferating islands of epithelial cells and these contained many melanosomes. However, few melanosomes were found in the epithelial cells. This study indicated a block in the transfer of pigment from the melanocyte to the basal epithelioma cells.

Several of the cultured epithelial cells were found to contain dense bundles of filaments resembling amyloid. It is likely that they are abnormal tonofilaments resulting from the differentiation and keratinization of basal cells *in vitro*.

Deeply pigmented basal cell epitheliomas are not uncommon and can be mistaken clinically for malignant melanomas. Light microscopic studies (Bloch, 1927; Eller & Anderson, 1933; Becker, 1933; Streitmann, 1959; Sanderson, 1961) have shown that these tumours contain functional melanocytes dispersed among the epithelial cells. Electron microscopic studies (Zelickson, Goltz & Hartmann, 1961; Zelickson, 1967; Woyke, Olszewski & Domagala, 1972) have shown that these melanocytes are active as regards melanogenesis and contain many melanosomes in various stages of development. Though in the stroma surrounding the epithelial tumour masses there were many melanin containing cells—melanophages—few melanosomes were to be found in the epithelial cells. These studies have indicated a block in the transfer of melanosomes from the melanocytes to the surrounding tumour cells. The purpose of this study was to investigate further the production, transfer and degradation of melanosomes not only in the tumour, but in primary cell cultures of pigmented basal cell epitheliomas.

MATERIALS AND METHODS

Three deeply pigmented basal cell epitheliomas from three patients were studied. A portion of each tumour was fixed in 10% formaldehyde saline and processed for light microscopy. Sections were cut and stained with haematoxylin and eosin, with a silver stain for melanin and with Congo red or methyl violet for amyloid.

For electron microscopy the tumours were chopped into small fragments not more than 1 mm^3 and then immediately fixed in 3% glutaraldehyde. These fragments were embedded in araldite, and ultrathin sections were cut and stained and later viewed in the electron microscope.

Primary cell cultures were made from two of these tumours. Portions of tumour were mechanically disaggregated to produce small fragments and a suspension of cells, after washing with Hanks BSS, was then seeded into Falcon flasks. For these primary cultures 199 medium was used to which was added 20% fetal bovine serum, penicillin 100 IU per ml and streptomycin 100 μg per ml. The nutrient medium was changed every 4 days. The colonies of proliferating cells were observed directly and by means of time-lapse photography. Cell cultures after 1 and after 3 weeks' growth *in vitro* were fixed with 3% glutaraldehyde and then embedded *in situ* in the flasks using Epon 812. Ultra-thin sections of selected colonies of cells were cut and stained and viewed in the electron microscope.

RESULTS

Tumours

Light microscopy of all three tumours showed proliferating islands of epithelial cells which in areas showed central cystic spaces and in others an adenoid appearance. At the periphery of these islands there was frequently a pallisading of cells. Sections stained with silver revealed many melanin containing cells, particularly at the periphery of the epithelial islands, and some of these were obviously dendritic. Melanophages were present in the dermal stroma surrounding the epithelial masses and contained large pigment granules that were also to be found in a few of the epithelial cells. Sections stained for amyloid showed several deposits in tumour cells and in the stroma.

On electron microscopy, 'light' and 'dark' epithelial cells were noted, which apart from the difference in the electron density of both their cytoplasm and nuclei, had the same fine structure. Few melanosomes were in these cells and these were mainly dispersed singly, though melanosomal complexes containing two or more melanized melanosomes were also present (Figs 1 and 2). Large paranuclear vacuoles containing fine granular material and cellular debris were found in some epithelial tumour cells sometimes resulting in an indentation of the nucleus to produce a crescentic deformity. A fine but quite distinct and continuous basement membrane separated the epithelial cells from the dermal stroma. The epithelial cells adjacent to the basement membrane showed hemi-desmosomes and also pinocytotic vesicles. The tonofilaments within the epithelial cells were often aggregated into bundles and normal looking desmosomes occurred between adjacent cells.

Melanocytes were to be found mainly at the periphery of the epithelial tumour bud and in relation to the basement membrane (Fig. 3). The cells were easily identified and seemed to be very numerous. They were markedly dendritic and contained melanosomes in various stages of development. The cells had a well developed Golgi with many vesicles. Melanosomes with arrays of melanofilaments with a periodicity of 9–10 μm were present but most of the melanosomes were fully melanized, their internal architecture obscured by the deposition of electron-dense melanin (Fig. 2). Autophagocytosis of melanosomes was noted with membrane bounded aggregates of degenerating granules in some of the melanocytes (Fig. 2). Centrioles indicating mitotic activity of the melanocytes were frequently seen (Fig. 3). Apart from melanocytes other dendritic cells were found including histiocytes and Langerhans cells.

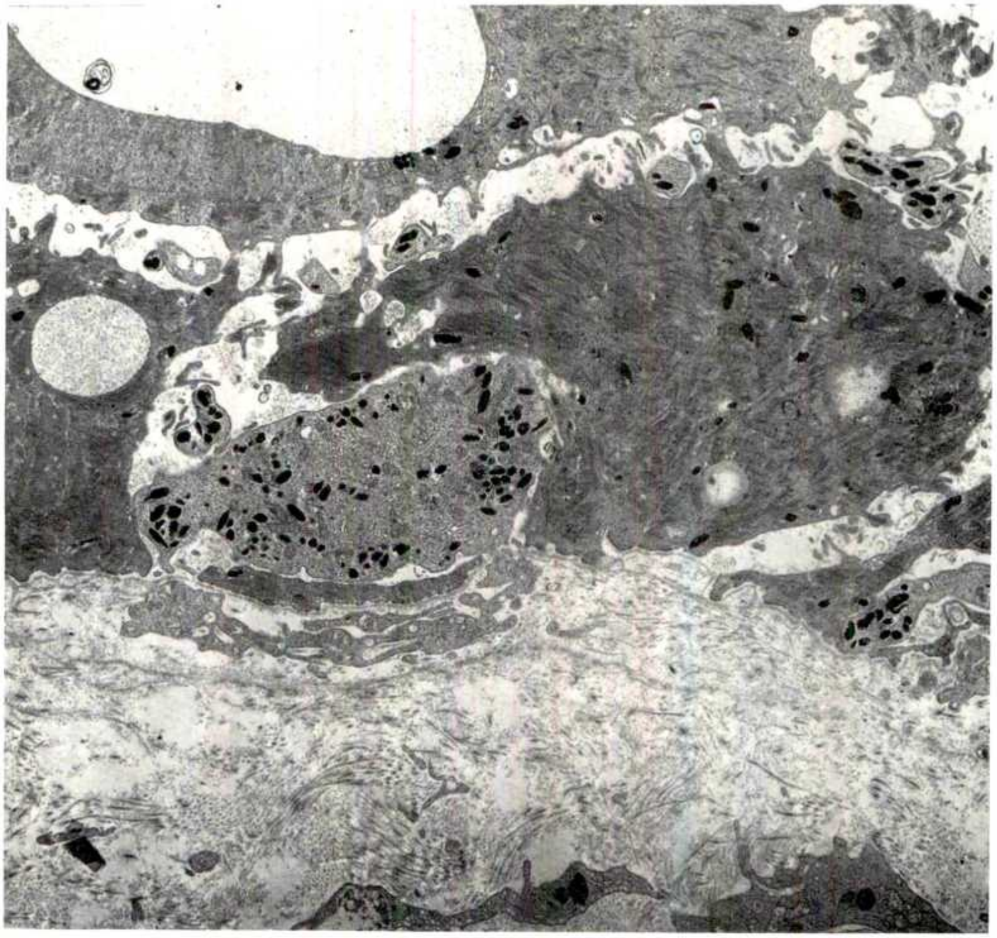

FIGURE 1. Electron micrograph showing portions of several melanocytes at periphery of epithelial tumour bud and containing many melanosomes. Few melanosomes in surrounding epithelial cells (× 5000).

Many melanophages were present in the dermal stroma and these contained many membrane bounded melanosomal complexes with variable numbers of melanosomes in various stages of degradation (Fig. 3).

Tissue cultures

Proliferating cell cultures were obtained from two tumours and were maintained successfully for over 2 months. Within 1 week many small colonies of cells had coalesced to form larger clumps with some overlap of the cytoplasm of the cells. Two types of cells could easily be identified (Fig. 4). One type, the majority, was epithelial in shape and these cells were closely adherent to each other with overlapping of their cytoplasm. This cell had a large central nucleus with often several nucleoli, and a granular cytoplasm. The nucleus of the cell was often in mitosis, particularly in the early growth phase of the primary cell cultures. The other cell was phase dense and smaller with obvious dendritic processes. The nucleus of the cell was small and eccentrically placed with a single nucleolus. Monolayers

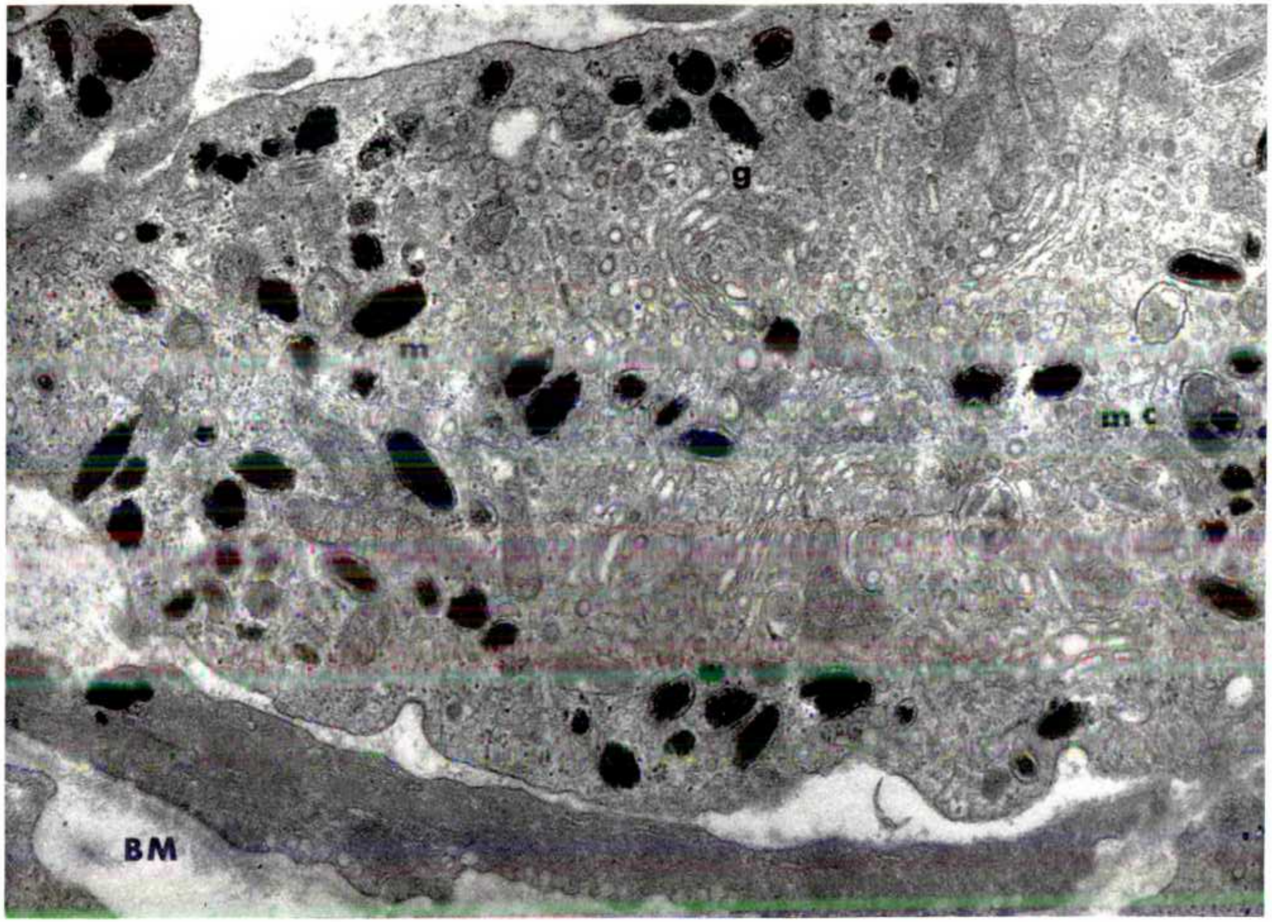

FIGURE 2. Higher magnification of portion of a melanocyte in Fig. 1 containing melanosomes (m) in various stages of development in relation to saccules and vesicles of the Golgi (g). Autophagocytosis of melanosomes to produce melanosomal complexes (mc). BM—basement membrane (× 26,000).

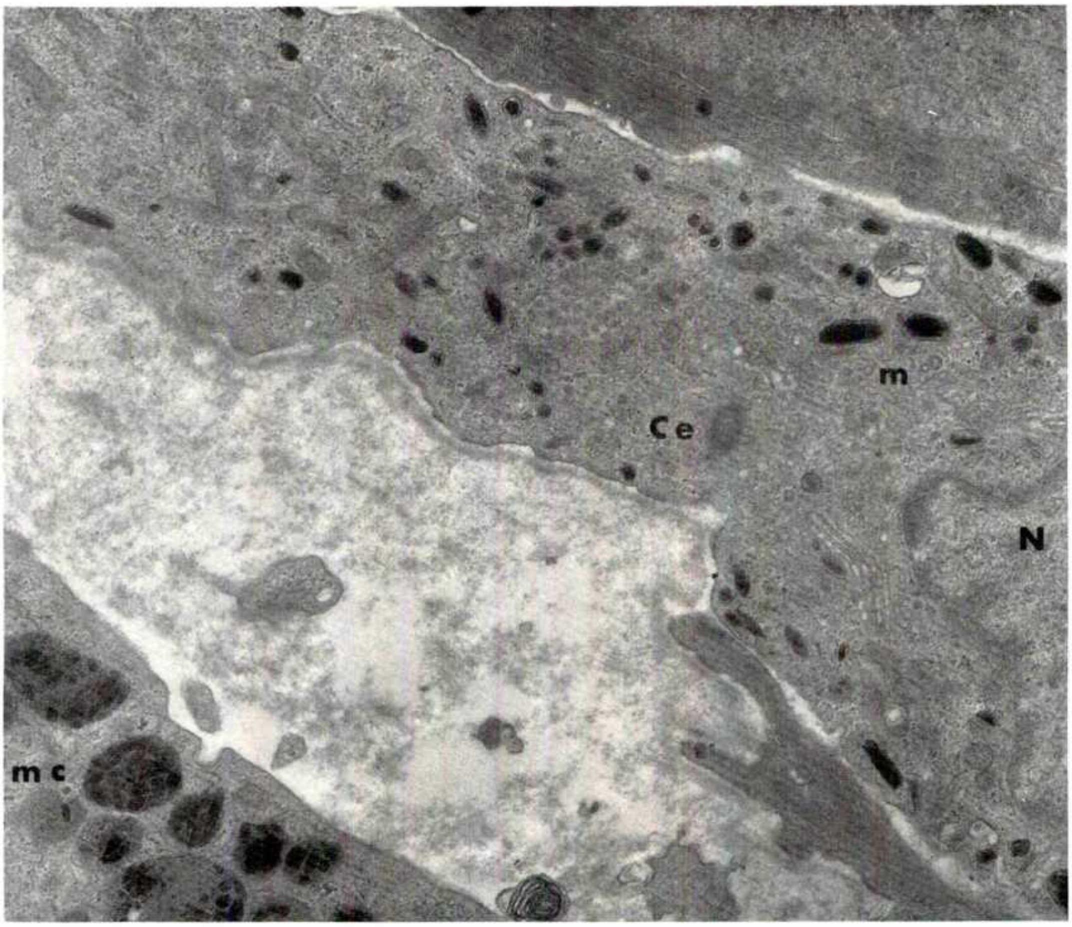

FIGURE 3. Portion of a melanocyte lying in relation to basement membrane and with a lobate nucleus (N), centriole (ce) and containing many melanized melanosomes (m). Large melanosomal complexes (mc) are present in a melanophage in dermal stroma (× 12,000).

incubated in dopa solution showed that these phase dense dendritic cells were dopa-positive and were melanocytes. Time lapse photography did not record the donation of pigment from these melanocytes to the surrounding epithelial cells though clumps of pigment granules were observed in the dendrites as well as in the epithelial cells. The dendrites made frequent contacts with the epithelial cells and it is likely that transfer of pigment took place *in vitro*.

Electron microscopy of cells grown in primary cultures for 1 and for 3 weeks showed that there was a considerable overlap of the cytoplasm of the epithelial cells which in some places were 3–4 cells deep. The cells were attached to each other by desmosomes (Figs 5 and 6) and filaments 5–7 μm in diameter that resembled tonofilaments were present in most of the cells. In some cells the filaments were very numerous and formed compact undulant bundles, especially in cells showing degenerative changes. The nuclei of the epithelial cells were large with multiple dispersed nucleoli.

Few melanosomes were found in the cultured epithelial cells. Those that were present were mainly solitary membrane bounded organelles with partially melanized melanofilaments (Fig. 5). Melanosome complexes were to be found in the epithelial cells, some consisting of large aggregates of melanosomes

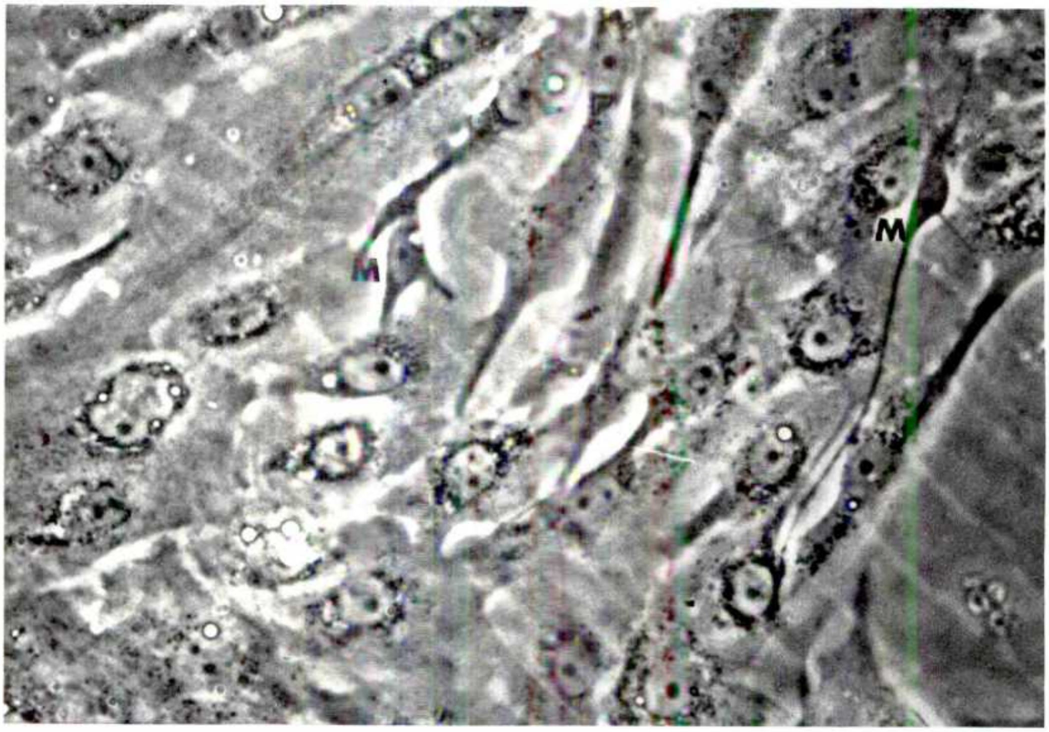

FIGURE 4. Basal cell epithelioma cells in culture at 21 days showing a few phase-dense dendritic cells—melanocytes (M) (×320).

set in a granular electron dense matrix and in various stages of degradation (Fig. 6). Several electron dense round bodies with double membranes were present and contained rod-shaped bodies resembling melanosomes. Especially in the older cultures, the majority of the epithelial cells contained vacuoles, lysosomal structures, lipid droplets and multi-vesicular bodies. Several cells contained masses of wavy bundles of filaments measuring 6–8 μm that occurred at the periphery near the cell membrane (Fig. 7) and resembled amyloid filaments.

Only a few melanocytes were found on electron microscopy of the tissue cultures. The melanosomes contained in the cytoplasm and dendrites were in various stages of development (Fig. 8). Their fine structure was essentially normal with filaments arranged in parallel or coiled and with a periodicity of particles along their length of 9–10 μm. These melanofilaments had a zig-zag pattern and cross linked. Most of the melanosomes seen were only partially melanized and were quite numerous in relation to saccules of the Golgi and the nucleus. The nucleus of the melanocytes was lobate and contained a large dispered nucleolus. Many elongated mitchondria were to be found in these cells.

Comment

Thirty per cent of basal cell epitheliomas contain pigment and as many as 10% are clinically pigmented (Winkelmann, 1972). Like pigmented seborrhoeic warts, the so called 'melanoacanthoma' of Mishima & Pinkus (1960), these tumours contain, in addition to the proliferation of epithelial cells, many functional melanocytes. Most of the pigment in these tumours appears to be in melanocytes and in macrophages lying in the dermal stroma surrounding the epithelial tumour buds. The

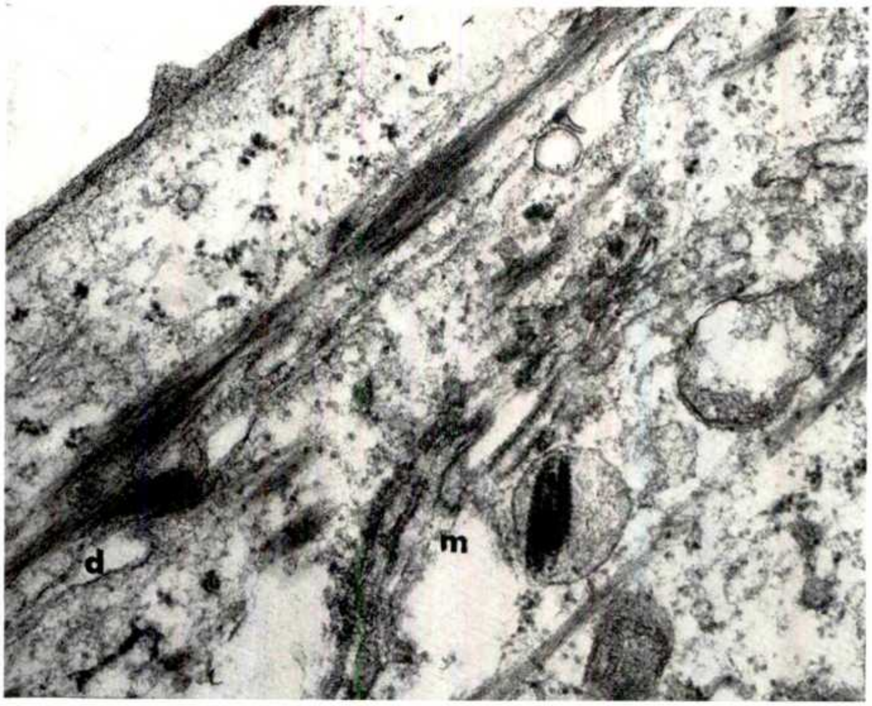

FIGURE 5. Electron micrograph of epithelial tumour cells, showing desmosomes (d) and solitary melanosome (m) surrounded by a well defined outer limiting membrane (×40,000).

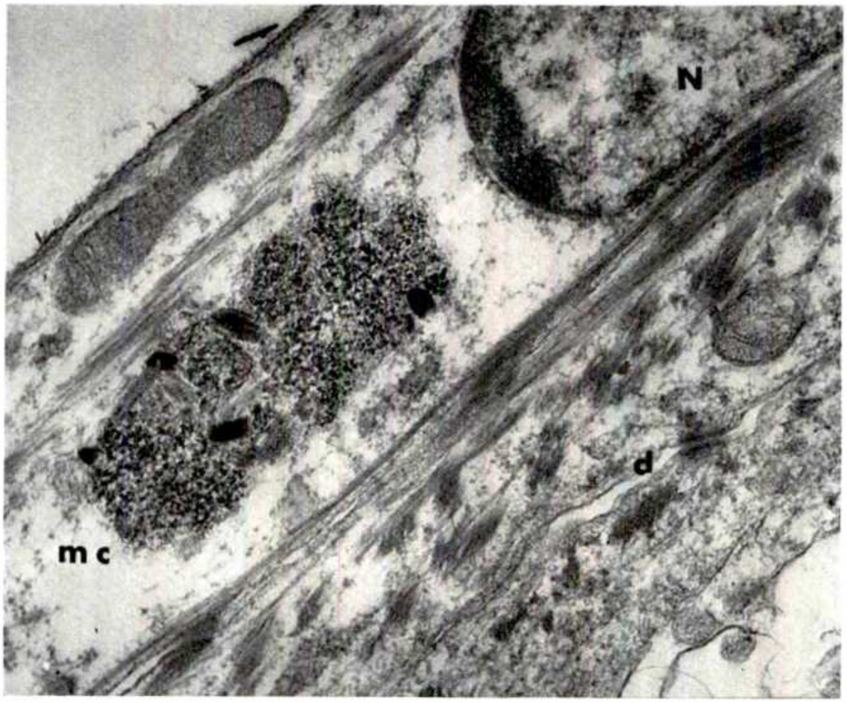

FIGURE 6. Tumour cell containing large conglomerate of melanosomes (mc). Well developed desmosomes (d) between epithelial cells containing bundles of tonofilaments. N—nucleus of cell (× 32,000).

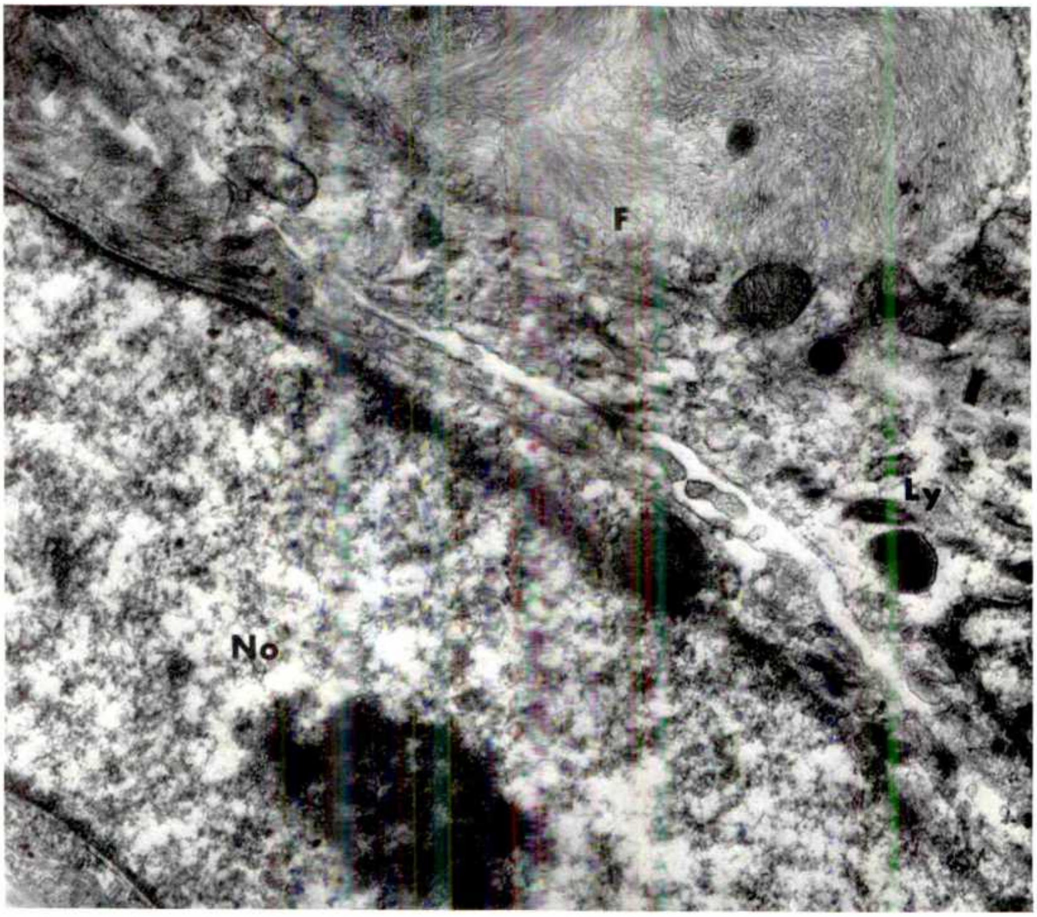

FIGURE 7. Wavy bundles of filaments (F) resembling amyloid at periphery of epithelial cell. Lysosomal structure (Ly). Dispersed nucleolus (No) (× 32,000).

pigmentation of the tumours in this study was variable, some areas in each tumour being more pigmented than others. What was striking was the relative lack of melanosomes in the epithelial tumour cells that surrounded the melanocytes and their dendrites. As noted by other workers (Zelickson, 1967; Woyke *et al.*, 1972) there seemed to be a block in the transfer of melanosomes to the basal epithelioma cells. Hashimoto & Lever (1968) and Ishibashi, Kasuga & Tsuchiya (1971) observed transferred melanosomes in the epithelial tumour cells often forming large conglomerates. In this present study few melanosomal complexes were found in the epithelial cells, but many were present in the numerous melanophages lying in the dermal stroma. Though the tissue culture studies showed no definite transfer of pigment from melanocytes to epithelial tumour cells, this undoubtedly occurred *in vitro*. The further differentiation of basal epithelioma cells in culture (Flaxman & Van Scott, 1968) may reverse the blockade and then permit the transfer of pigment. Even so, only a few melanosomes were to be found in the epithelial cells and were partially melanized and mainly appeared to be degraded. The breakdown of melanosomes by lysosomal enzymes occurs in keratinocytes and this is likely to be taking place in the epithelial tumour cells *in vitro*. Numerous melanophages were present in the dermal stroma whose pigment almost certainly originated from melanin containing

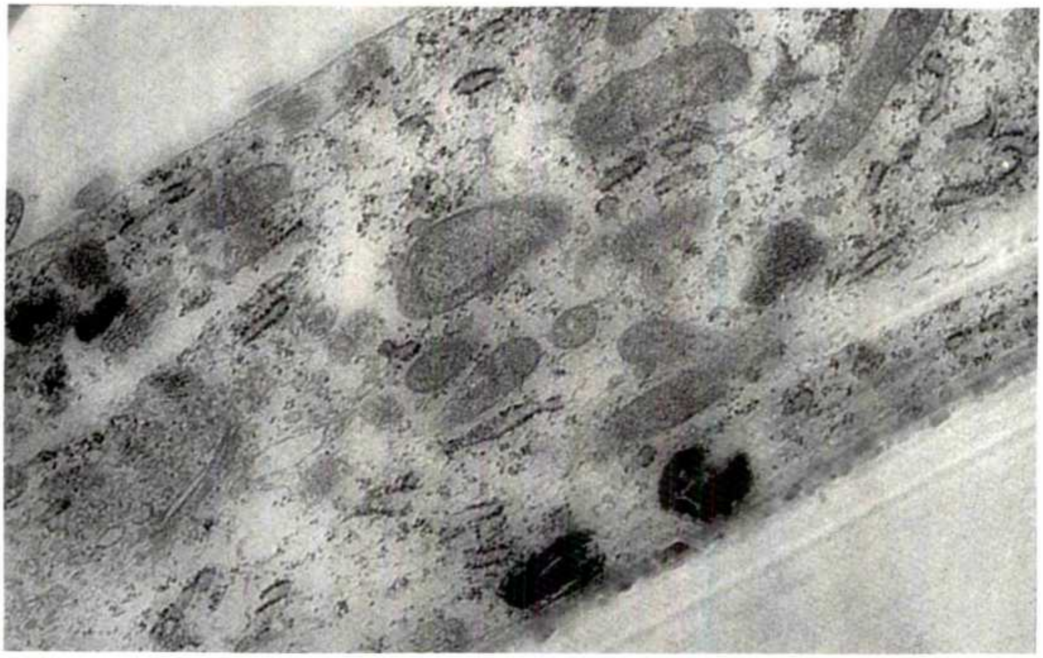

FIGURE 8. Portion of melanocyte containing partially melanized melanosomes (× 32,000).

cells in the epithelial tumour buds. On disruption or death of pigment laden cells in the tumour buds, phagocytosis of the debris including the melanosomes takes place. Macrophages, probably the histiocytes seen in the tumour epithelium, remove this pigment and then migrate back to the dermal stroma. The melanophages contained numerous melanosomal complexes and other cellular debris in lysosomal structures.

In stained sections of one of the tumours small islands of amyloid were found in the dermal stroma. Amyloid-like filaments were present in epithelial tumour cells grown for 3 weeks in tissue culture. These filaments resembled dense aggregations of tonofilaments and probably resulted from the differentiation and keratinization of basal epithelioma cells that occurs *in vitro* (Flaxman & Van Scott, 1968). Amyloid-like material has been found in the stroma of basal cell epithelioma (Genter & Beurlet, 1965; Hashimoto & Brownstein, 1973) and in other epithelial tumours (Hashimoto & King, 1973). The way this material is formed is still uncertain but could be the result of an interaction between abnormal epithelial cells and fibroblasts. Black & Wilson Jones (1971) thought that the epidermis played an important part in the histogenesis of macular amyloidosis.

This study confirms those of Zelickson (1961, 1967) that there is a relative block in the transfer of pigment from melanocytes to the epithelial tumour cells. Perhaps further *in vitro* studies using latex particles the size of melanosomes (Wolff & Konrad, 1972) may show that the epithelial tumour cells are unable to phagocytose these granules.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the Medical Research Council. I wish to thank Miss Jennifer Senior and Miss Lisa Hartley for their technical assistance and Dr R. Fountain who referred one of the patients.

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British Journal of Dermatology (1975) **93**, 371

Pigmentary demarcation lines

COMPARISON OF NEGROES WITH JAPANESE

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Accepted for publication 21 January 1975

SUMMARY

The natural pigmentary demarcation boundaries of the skin can be classified as: Group A—lines along the upper limb with variable trans-pectoral extension; Group B—lines along the lower limb; Group C—paired lines in median or paramedian course on the chest with midline abdominal extension; Group D—posteromedian demarcation; and Group E—bilaterally symmetrical, obliquely oriented hypopigmented macules on the chest. Groups A and C are the most distinct and occur in both Negroes and Japanese.

In 1913, Matzumoto, who studied Japanese, provided a detailed description of the pigmentary demarcation line of the upper limb and its occasionally visible trans-pectoral extension, and that along the lower limb. Miura's classification (1951, 1952) has been extended in the Table to include additional findings in Negroes (Fig. 1 and Table 1).

Group A anterior brachial demarcation among Negroes was described in 1938 by Futcher, who cited the anthropological tenet that in general dorsal and extensor surfaces of the body are more pigmented than ventral and flexor aspects. Futcher recognized on the upper limbs of some Negroes an abrupt transition at the junction of darker posterolateral and lighter anteromedial skin. A distinct antero-brachial line of pigmentary demarcation was found by him bilaterally in thirty-five of 200 Negroes (17·5%) and unilaterally in four more (2%). Neither age, which ranged in Futcher's series from 11 to 74 years, nor sex, nor overall darkness of the Negro skin seemed significantly to affect the frequency of the line.

Vollum (1972) observed linear demarcation of pigmentation along the anterior brachial surface of 26% of 235 Negro children from the West Indies. In 3% the line was unilateral. The lines were most evident along the upper portion of the arm; proximally they faded over the head of the humerus; distally they continued to the lateral side of the base of the first metacarpal, though often indistinct below the elbow. Occasionally they were seen along the upper-posterior aspect of the arm corresponding to the dorsal axial line.

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In a preliminary survey of our own at Metropolitan Hospital, New York City, antero-brachial demarcation was discernible in thirty-seven among 100 consecutive Negroes, twenty of fifty-two males (38%) and seventeen of forty-eight females (35%) (Fig. 2, A), about half the population being of partial Spanish extraction. The demarcation was perceptible in eight of the twenty-six subjects 12 years of age and younger (31% of this group) and in twenty-nine of seventy-four persons (39%) aged from 13 to 72 years. Thus, as suggested by Vollum (1972), there seems to be a slight tendency, though statistically not significant, for this demarcation to be more apparent with advancing age. Other factors reported as emphasizing the appearance of brachial demarcation lines include emaciation with relaxation of the skin (Futcher, 1938) and, in Japanese, various disease states but especially pleurisy and pulmonary tuberculosis (Miura, 1952).

As outlined in Table 1, trans-pectoral extension of Group A in several variations has been observed among Japanese. We fortuitously observed an occasional Negro with trans-pectoral extension of Type I (Fig. 3, A). It is not known with what frequency trans-pectoral extension is found in Japanese and Negroes.

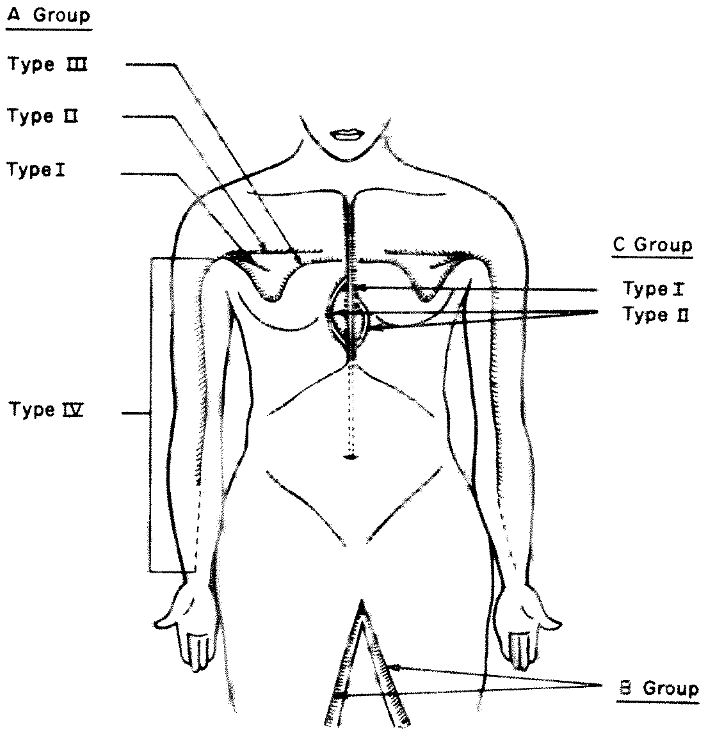

FIGURE 1. Demarcation lines of pigmentation (feathered and hyphenated) found in Japanese, after Miura. (Ventral axial lines of inner thigh, to which Group B may correspond, are usually shown in posterior view.)

Ito (1965) reported that the antero-brachial pigmentary demarcation line in Japanese was seen only in 4·3% and was ten times commoner in females. The pigmentary demarcation line of the posterior aspect of the arm is rarely found in Japanese (Miura, 1952) and Negroes (Vollum, 1972).

Group B demarcation, which was described among Japanese (Matzumoto, 1913), was not reported in Negroes by Futcher (1938) or Vollum (1972). Examination of lower limbs was not routinely performed in the present survey but in one subject with prominent brachial lines we noticed a pigmentary demarcation line along the lower lateral portion of his leg.

Group C lines in Japanese correlate with the 'mediosternal line,' a longitudinal hypopigmented band of variable width on the chest which is discernible in more than one-third of Negroes (Kisch & Nasuhoglu, 1953; Selmanowitz & Krivo, 1973). This band (or when narrow, a line) results from two side-by-side pigmentary demarcation lines (Figs 1 and 2 C).

Within Group C, Miura recognized Types I and II among Japanese which we found as well as Types III and IV in Negroes. In both Negroes and Japanese there may be extension of Group C lines along the abdominal midline, giving the appearance of a hypopigmented line overlying the linea alba.

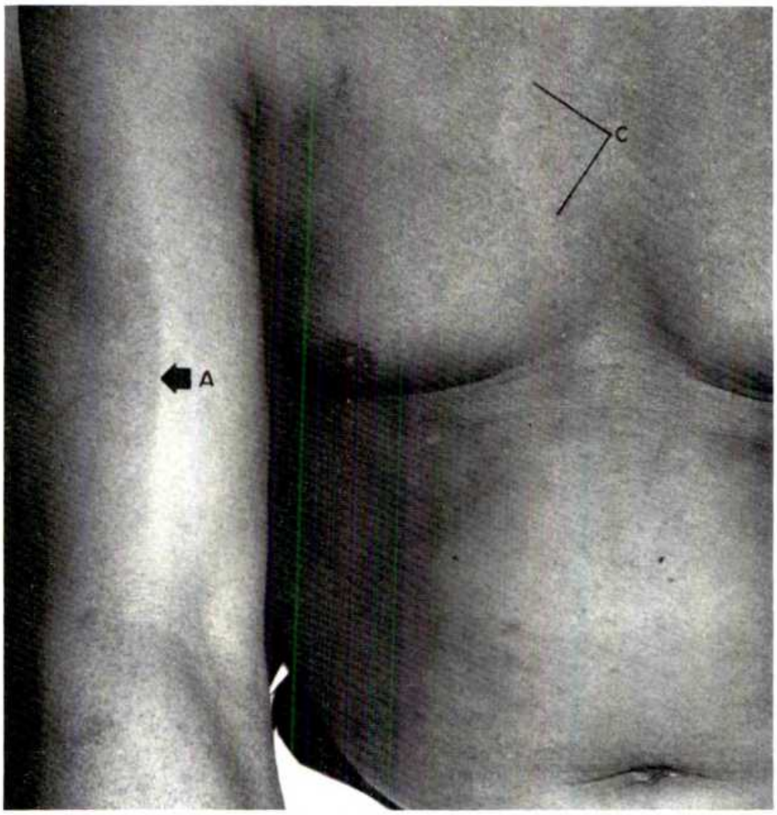

FIGURE 2. (A) Pronounced brachial demarcation line of pigmentation (Group A) in typical course. (C) Type II mediosternal line (Group C) arches from xiphisternal midline and returns to midline superiorly.

Group D, posteromedian demarcation, is rare among Japanese (Miura, 1951). We have not yet examined Negroes for this demarcation.

Group E markings, which are not too uncommon on the chests of Negroes (Selmanowitz & Krivo, 1973), are slightly hypopigmented streaks, wider bands, or lanceolate areas that should be differen-

TABLE 1. Pigmentary demarcation lines (Japanese and Negroes)

Group	Location most apparent	Other locations or extensions	Types	Apparency with increasing age	Possible correspondence
A Upper limb —pectoral	Anterior brachial	Anterior forearm Upper border axilla Trans-pectoral Posterior brachial	I. Downward trans-pectoral slope from upper border axilla towards midline chest II. Horizontal trans-pectoral III. Wavy trans-pectoral IV. Proximal termination upper border axilla	Unchanged or increased (emphasized with cachexia and in Japanese with TB)	Axial lines (ventral and dorsal)
B Lower limb	Inner thigh (mid-perineum to inner border popliteal fossa)	Spirals down leg Extension to inner ankle or to lateral malleolus		Unknown	Axial lines
C 'Mediosternal line'	Pre- and para-sternal	Abdominal midline	I. Longitudinal midline (anteromedian) II. Arched (Figs. 1 and 2) III. Curves from midline near suprasternal notch IV. Wavy; arches widely from xiphisternal region along upper contour breast; returns midline at base of neck	Less (reported in Negroes)	Midsaggital plane; nerve terminations or transmedian overlap; linea nigra
D Posteromedian	Spinal	Rare		Unknown	Midsaggital plane; nerve terminations or transmedian overlap
E Bilateral chest markings	Within zone from mid-1/3 clavicle to periareolar skin		I. Subclavicular II. Approximately midway between mid-clavicle and nipple III. Periareolar	Less	Unknown

tiated from 'ash-leaf' hypopigmented macules of tuberous sclerosis (Fitzpatrick *et al.*, 1971). They tend to be bilaterally symmetrical and obliquely oriented (Table 1 and Fig. 3).

From a distance the pigmentary demarcations appear linear but close inspection reveals many fine indentations (or 'feathering'). Comparing skin on either side of a demarcation line, no clinical differences have been recognized in hair growth, sweating or sensation. Matzumoto (1913) and Miura (1951) reported slightly increased epidermal melanization on light microscopy on the appropriate side.

Aetiology

Miura (1951) considered the pigmentary demarcation lines as being atavistic. Matzumoto (1913) postulated that the dorsally more pigmented skin of animals serves for protection against ultraviolet rays and for thermoregulation. In both Japanese and Negroes the possibility has been raised that the various lines and markings are dominantly inherited (Ito, 1965; Weary & Behlen, 1965; Selmanowitz & Krivo, 1973).

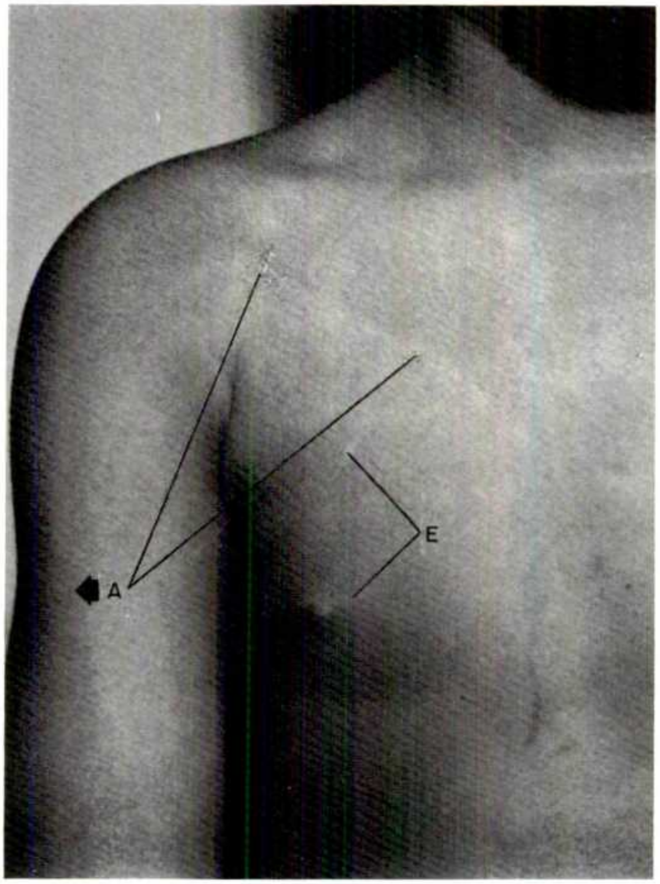

FIGURE 3. (A) Anterior brachial pigmentary demarcation line with its wedge-like extension (unilateral in this patient) over the axilla and downwards towards midline (Group A, Type I). (E) Obliquely oriented hypopigmented macules (Group E, Types II and III). Corresponding macules found on side not shown.

Miura (1951) indicated that the pigmentary demarcation lines coincide with the marginal lines of cutaneous nerve distribution: Groups A and B with the axial lines; Groups C and D with median or paramedian terminations of peripheral nerves. According to Vollum (1972) the pigmentary demarcation line on the anterior surface of the forearm in Negro children passes lateral to the axial line. However, one cannot be dogmatic about precise courses of axial lines. Maps of the boundaries of individual dermatomes and of the courses of axial lines determined by different methods vary considerably (Jarrett, 1973), and there is individual variation (Schliack, 1969).

Voigt's boundary lines, the lines which delimit the distribution of peripheral nerves (Arey *et al.*, 1957), are generally indeterminate because of the neural-sensory overlap between dermatomes. However, such overlap is lacking across the axial lines (Jarrett, 1973). Group A anterior demarcation has been referred to as 'Voigt's line' (Ito 1965; Vollum, 1972) but more correctly it should be considered as possibly corresponding to *one* of Voigt's lines in axial course.

The Group C mediosternal line occurs where transmedian overlap of nerves is often suggested clinically (Jarrett, 1973) and where dorsoventral migration of melanoblasts from the neural crest may terminate (Rawles, 1948; Mintz, 1967). Group E markings are located over fields where the overlap of nerves across dermatomes develops extensively (Ranson & Clark, 1959).

Further Significance

The pigmentary demarcations indicate a duality in the population of melanocytes which may be related to underlying neural influence and perhaps to actinic stimulation which, at least phylogenetically, would be more pronounced over the more exposed extensor surfaces.

Various dermatoses correspond by distribution to the marginal lines of cutaneous innervation associated with the pigmentary demarcation lines. Ito (1965) described a case of extensive vitiligo in a Japanese which was sharply bounded in correspondence with Group A and C lines. We have seen the same distribution of vitiligo in Caucasoids. Montgomery (1901) speculated on the distribution of linear naevus verrucosus in relationship to Voigt's lines, courses of cutaneous nerves, metameric lines, and lines along the embryonic sutures which follow the trend of growth of the tissues. Selmanowitz (1970) described unilateral dermatomal distribution of telangiectases where axial lines separated involved skin from the normal, in a manner reminiscent of the mapping of dermatomes by the hyperaemic zones resulting from electrical stimulation of posterior nerve roots (Schliack, 1969).

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Absorption and biotransformation of topically applied 8-(methylthio)cyclic AMP

STUDIES IN DOGS AND HUMANS

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Accepted for publication 30 March 1975

SUMMARY

8-(Methylthio)cyclic AMP-^{35}S was applied topically to the intact or abraded skin of dogs at a dose of about 2·5 mg/kg. In this species, an average of 7% of the dose was absorbed through the intact and 62% through abraded skin. Half-lives for radioactivity in plasma averaged 3·6 h. After the topical application of the drug to the intact skin of dogs, the average concentration in plasma of glucose, but not of insulin, was increased by as much as 21%; after application to abraded skin, glucose and insulin were increased, on average, by as much as 84 and 221%, respectively. 8-(methylthio)adenosine and two unidentified compounds were present as metabolites in the urine of dogs; some unchanged 8-(methylthio)cyclic AMP-^{35}S was also excreted. 8-(methylthio)cyclic AMP-^{35}S was also applied topically (25 mg; 0·32 mg/kg) to the intact and stripped skin of normal human subjects under conditions similar to those used for dogs. Three subjects with intact skin did not absorb any of the drug, whereas three subjects with stripped skin absorbed 0·5, 8·3, and 23·3% of the dose. The half-life of radioactivity in the plasma of the subject with the greatest absorption was 0·5 h during the first 2 h, and 11 h for the next 10 h. During the first 2 h, this same subject excreted unchanged drug and 8-(methylthio)adenosine in his urine. No changes in glucose or insulin concentrations in plasma were observed in any of the subjects nor was there any apparent irritation of the skin.

Cyclic AMP has been considered to be a biochemical mediator of abnormal epidermal proliferation (Voorhees *et al.*, 1973). Specifically, in psoriasis, concentrations of cyclic AMP in the plaques have been reported to be significantly lower than those in healthy skin (Voorhees *et al.*, 1972a), although reports to the contrary have also appeared (Härkönen, Hopsu-Havu & Raij, 1974). Voorhees *et al.* (1972b) proposed that the topical application of cyclic AMP or one of its biologically active analogues might prevent or ameliorate the psoriatic condition. 8-(methylthio)cyclic AMP-^{35}S (Fig. 1) is an analogue of cyclic AMP that has exhibited biological activity in the stimulation of a purified cyclic

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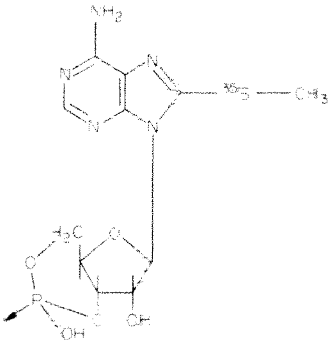

FIGURE 1. Structure of 8-(methylthio)cyclic AMP-^{35}S.

AMP-dependent protein kinase (Muneyama *et al.*, 1971), the inhibition of cyclic nucleotide phosphodiesterase from cat heart and rat brain (Harris, Phillips & Goldenberg, 1971; Harris *et al.*, 1973), and the activation of tyrosine transaminase from hepatoma cells grown in culture (Van Wijk, Wicks & Clay, 1972). 8-(methylthio)cyclic AMP has also been shown to stimulate the production of glucose and insulin in the plasma of dogs after oral or intravenous administration (Dreyfuss *et al.*, 1975). The present studies were conducted to evaluate the percutaneous absorption of 8-(methylthio)cyclic AMP-^{35}S and some of its biological effects in dogs and in normal human subjects. The biotransformation of the drug in each species was also studied.

METHODS

Studies in Dogs

Pure-bred beagles, weighing 9·4–11·2 kg, were used in these studies. Animals were housed in metabolic cages that permitted the separate collection of urine and faeces. 8-(methylthio)cyclic AMP-^{35}S had an initial specific activity of 12·1 μCi/mg in the undiluted state; no radiochemical impurities were detectable.

Dogs given the drug topically had the hair shaved from a portion of the back the day before drug application; the skin was washed with a mild soap and allowed to dry. On the day of drug application, a 5 × 6 cm area of skin was outlined with adhesive tape. Fine sandpaper was used to abrade the skin, when such treatment was required. Abrasion was continued until the skin had just begun to appear slightly raw. 8-(methylthio)cyclic AMP-^{35}S (25 mg in 0·5 ml of a 5% aqueous ammonium tartrate formulation, pH 5·5) was applied to the skin by spreading the preparation uniformly over the area with a glass rod. The small amount of radioactivity remaining on the glass rod was determined by placing the rod in 0·1 M Na_2CO_3 and counting the solution. The amount of radioactivity remaining on the rod was unavailable for absorption and was subtracted from the theoretical dose to give the actual dose. The area on which the dose was applied was immediately occluded with plastic wrap that was then fastened with more tape to the outlining adhesive tape. The plastic wrap was covered with a large gauze pad which was held in place by winding an elastic bandage several times around the dog's body. A protective collar was placed around the dog's neck to prevent the animal from removing the elastic bandage. Twelve hours after dosing, the occlusive wrap and the outlining tape were removed. The area of skin on which the drug had been applied was wiped three times with separate pieces of cotton wool that had been moistened with 0·1 M Na_2CO_3. The cotton wool and occlusive wrap were placed together in 0·1 M Na_2CO_3, and extracted by shaking, then a portion of the extract was counted.

Additional studies were conducted in dogs, at a later time, to account better for all of the radioactivity applied to the skin. The same methodology described above was used to apply the 8-(methylthio)cyclic AMP-^{35}S to the skin, but the dogs were sacrificed at 2 and 7 days after they had been dosed. The skin to which the drug had been applied, with any adhering subcutaneous fat, was excised, homogenized in 0·1 M Na_2CO_3 and counted. A homogenate of the paper tissues used to remove the drug, 12 h after dosing, and of the occlusive wrap was prepared in 0·1 M Na_2CO_3 and counted. Finally, the adhesive tape used to outline the skin and to secure the occlusive wrap was extracted by shaking with 0·1 M Na_2CO_3, and counted.

Studies in humans

Two groups of normal male subjects (three per group) gave their informed consent to participate in this study. One group of subjects was given a single topical application of 8-(methylthio)cyclic AMP-^{35}S (25 mg; 42 μCi) on a 3×5 cm area of shaved but otherwise normal skin, outlined by tape on the volar side of one forearm. The drug was supplied as a 5% solution in sterile ammonium tartrate (pH 5·5). The other forearm of each subject was treated only with the ammonium tartrate vehicle. In this way it could be determined whether any irritation had occurred to the skin due to the presence of the 8-(methylthio)cyclic AMP. Each preparation (0·5 ml) was applied dropwise to the outlined area of either arm, with partial drying between applications, until the entire amount had been applied. Then the partially moist areas of skin were immediately occluded with plastic wrap for 12 h by taping the wrap to the outlining tape on each arm. After 12 h, the occluding wrap and outlining tape were removed and analysed for the presence of radioactivity, as described above for the studies with dogs. In addition, the skin within and around the area to which the dose of 8-(methylthio)cyclic AMP-^{35}S had been applied was wiped at least twice with cotton wool that had been moistened with the ammonium tartrate vehicle; these samples were also counted. The other group of subjects received the same treatment as the first group, except that, 1 h before dosing, the keratin layer of the skin of these subjects was stripped by repeated applications of adhesive tape, until the skin in the outlined area had become moist and glistened in reflected light. Concentrations of glucose in plasma were determined 0·5, 4, 12, 24, 48 and 96 h after dosing. Concentrations of insulin and glucagon were determined by radioimmunoassay 0·5, 4 and 12 h after dosing.

Assay of radioactive samples

Samples of urine and solutions of 0·1 M Na_2CO_3 were counted directly in 15 ml of scintillator (Bray, 1960).

A 0·4 ml sample of plasma was dissolved in 2 ml of NCS solubilizer (Amersham/Searle). The sample was counted with 15 ml of Bray's or toluene scintillation fluid. This scintillation fluid contained, per litre of toluene, 5 g of 2,5-diphenyloxazole and 0·3 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene.

Faecal samples were homogenized in about three volumes of methanol. A 100-mg sample of the homogenate was digested in 1 ml of NCS solubilizer for 16 h with shaking and was then bleached with 30% hydrogen peroxide. The sample was counted with 15 ml of scintillator (Bray, 1960).

Samples of dog skin were homogenized in 0·1 M Na_2CO_3, then an aliquot was solubilized in 2 ml of NCS solubilizer and counted in 15 ml of toluene scintillation fluid.

Counting of samples

Radioactivity in each sample was measured by a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3380. Counting efficiency was determined with automatic external standardization and the use of previously prepared quench curves.

Chromatography

Samples of urine were spotted on plates of cellulose Q2-F (Quantum Industries, New Jersey) and developed in one or more of the solvent systems: (1) isopropanol–28% ammonia–water (7:1:2); (2) n-butanol–pyridine–glacial acetic acid–water (30:20:6:24); and (3) 95% ethanol–EDTA buffer (70:30). (The EDTA buffer was prepared by mixing 1 M ammonium acetate and 3×10^{-3}M ethylenediaminetetra-acetate (EDTA) and adjusting the pH to 5·0 with glacial acetic acid.) Chromatograms were scanned for the presence of radioactivity with a Nuclear Chicago Actigraph III scanner. Areas of the various radioactive peaks were integrated by the use of a planimeter.

Glucose concentrations in the plasma of dogs

These analyses were performed with a Beckman Autoanalyzer, Model DSA-560.

Insulin concentrations in the plasma of dogs

These analyses were performed with an Insulin Radioimmunoassay Kit (Amersham/Searle). The results were calculated from standard curves that had been prepared from standardized human insulin.

RESULTS

Studies in dogs

Concentrations of radioactivity in the plasma of dogs after the topical application of 8-(methylthio)-cyclic AMP-^{35}S to either intact or abraded skin are shown in Fig. 2. After drug application to intact skin, maximum concentrations of radioactivity were present in the plasma 1–1·5 h after dosing and declined slowly thereafter. After drug application to abraded skin, maximum concentrations of radioactivity in plasma were attained 15 min after dosing and declined with an average half-time of about 3·6 h during the interval of 1·5–8 h. The amounts of radioactivity absorbed after application to either the intact or abraded skin could be calculated by integrating the areas under the curves for plasma concentrations after topical or intravenous dosing, the latter route having been studied earlier (Dreyfuss *et al.*, 1975). These calculations indicated that an average of 6·3 and 65·1% of the dose had been absorbed through intact and abraded skin, respectively.

Concentrations of glucose and insulin in plasma were determined for these same dogs, as shown in Table 1. For the dogs dosed on intact skin, there was, on average, as much as a 21% increase in the glucose concentration ($P < 0·01$), but this relatively small hyperglycaemic effect is apparently not large enough to trigger an increase in the production of insulin. For the dogs dosed on abraded skin, concentrations of glucose increased rapidly during the first few minutes after dosing and concentrations of insulin increased in a manner that generally paralleled those found for glucose. Although increases in glucose were, on average, 84%, increases in insulin, on average, were as much as 221%.

Table 2 shows the excretion of radioactivity in the urine and faeces of dogs after dosing on either intact or abraded skin. On average, a total of 7·3 and 58·9% of the dose was excreted during the 5 days. Most of the radioactivity, regardless of the condition of the skin, was excreted in urine during the first 24 h after dosing. The average amounts of total radioactivity excreted by dogs agree well with the amounts calculated from the areas under the curves for the concentrations of radioactivity in the plasma of dogs that had been dosed topically or intravenously. Thus, for intact skin, 7·3% of the dose was excreted in urine and faeces, whereas, based on concentrations of radioactivity in plasma, 6·3% had been absorbed. For abraded skin, 58·9% of the dose was excreted in urine and faeces, whereas, based on concentrations of radioactivity in plasma, 65·1% had been absorbed.

The urine of dogs collected during the first 24 h after they had been dosed on abraded skin was

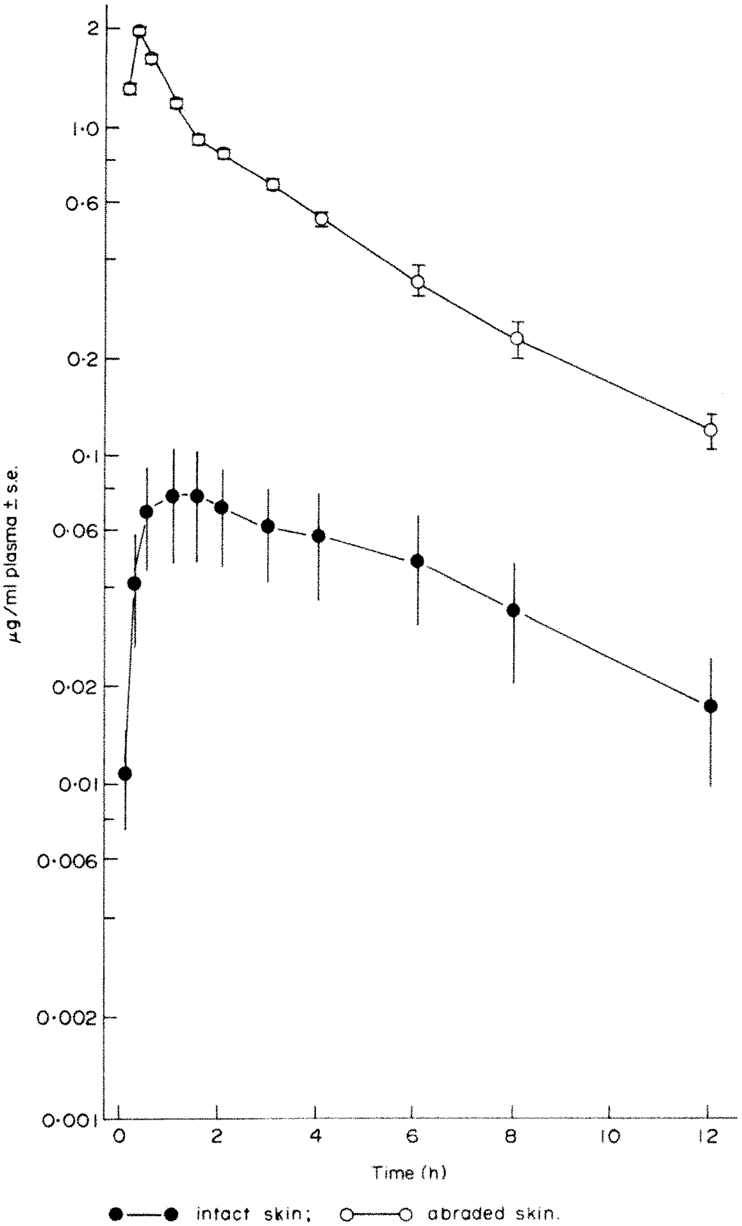

FIGURE 2. Average concentrations of radioactivity in the plasma of dogs after the topical administration of 2·5 mg/kg doses of 8-(methylthio)cyclic AMP-^{35}S to either the intact or abraded skin of dogs. The same two male and two female dogs were used in both phases of the test, but the compound was applied first to intact skin and later to abraded skin.

chromatographed in solvent system 1. These chromatograms showed the presence of four radioactive components, very similar to those that had been found earlier in the urine of dogs dosed intravenously (Dreyfuss *et al.*, 1975). Of the four components found, one was unchanged 8-(methylthio)cyclic AMP-^{35}S, a second was 8-(methylthio)adenosine, and the other two have not been identified.

To account better for all the radioactivity applied to the intact skin of dogs, another group of

TABLE 1. Concentrations of glucose and insulin in the plasma of dogs after the topical administration of 8-(methylthio)cyclic AMP-^{35}S (2·5 mg/kg) to either intact or abraded skin

Time (min)	Intact skin*		Abraded skin*	
	Glucose (% change ± s.e.)	Insulin (μunits/ml ± s.e.)	Glucose (% change ± s.e.)	Insulin (μunits/ml ± s.e.)
0	(0)	19 ± 4	(0)	24 ± 1
5	10·0 ± 5·5	15 ± 4	60·3 ± 26·4	35 ± 3
15	13·5 ± 5·0	16 ± 2	84·0 ± 10·4	77 ± 2
30	21·0 ± 5·6	17 ± 2	70·3 ± 21·6	67 ± 10
60	20·8 ± 2·7	15 ± 2	49·8 ± 16·2	35 ± 5
90	20·8 ± 5·2	—	54·2 ± 23·4	—
120	20·3 ± 5·1	—	18·8 ± 6·3	—
180	16·5 ± 6·2	—	4·3 ± 5·4	—
240	17·0 ± 6·4	—	−4·8 ± 4·8	—

* The same two male and two female dogs were used in both phases of the test, but the compound was applied first to intact skin and later to abraded skin.

four dogs was given topical 2·5 mg/kg doses of 8-(methylthio)cyclic AMP-^{35}S. The animals were sacrificed 2 or 7 days after they had been dosed and the skin to which the drug had been applied was excised and analysed, as were the materials used to apply and occlude the drug (Table 3). Radioactivity was still present in the skin 2 and 7 days after dosing (ranging from 15 to 33%). Some radioactivity was present on the paper tissues used to wipe off the drug 12 h after dosing, and on the occluding wrap. Most interesting were the substantial amounts of radioactivity present on the adhesive tape (ranging from 42 to 65%), indicating that some of the aqueous tartrate vehicle, containing the drug, is absorbed by the adhesive tape. The skin of the dogs that were sacrificed 2 days after they had been dosed was homogenized and a portion of the homogenate was chromatographed, as shown in Fig. 3. The major amount of radioactivity in both samples of skin was still unchanged 8-(methylthio)-cyclic AMP-^{35}S (49 and 64%) and significant amounts of 8-(methylthio)adenosine (25 and 32%).

Studies in humans

None of the subjects who had drug applied to intact skin had any measurable concentrations of radio-

TABLE 2. Excretion of radioactivity by dogs after the topical administration of 8-(methylthio)cyclic AMP-^{35}S to either intact or abraded skin (2·5 mg/kg)

Condition of skin	Average % of dose ± s.e.		
	Urine	Faeces	Total
Intact*	4·85 ± 1·72	2·46 ± 0·42	7·31 ± 1·97
Abraded*	43·83 ± 2·75	15·03 ± 1·49	58·85 ± 2·89

* The same two male and two female dogs were used in both phases of the test, but the compound was applied first to intact skin and later to abraded skin. Excreta were collected for 5 days.

TABLE 3. Recovery of radioactivity after the topical application of 8-(methylthio)cyclic AMP-^{35}S (2·5 mg/kg) to the intact skin of dogs

	% of dose			
	After 2 days		After 7 days	
	Dog Q2-259 (male)	Dog Q2-267 (female)	Dog Q2-260 (male)	Dog Q2-268 (female)
Skin	32·57	14·97	13·57	26·25
Wipes and occluding wrap	8·75	19·94	14·56	34·52
Adhesive tape holding occluding wrap	51·53	64·87	64·13	42·30
Total	92·85	99·78	92·26	103·07

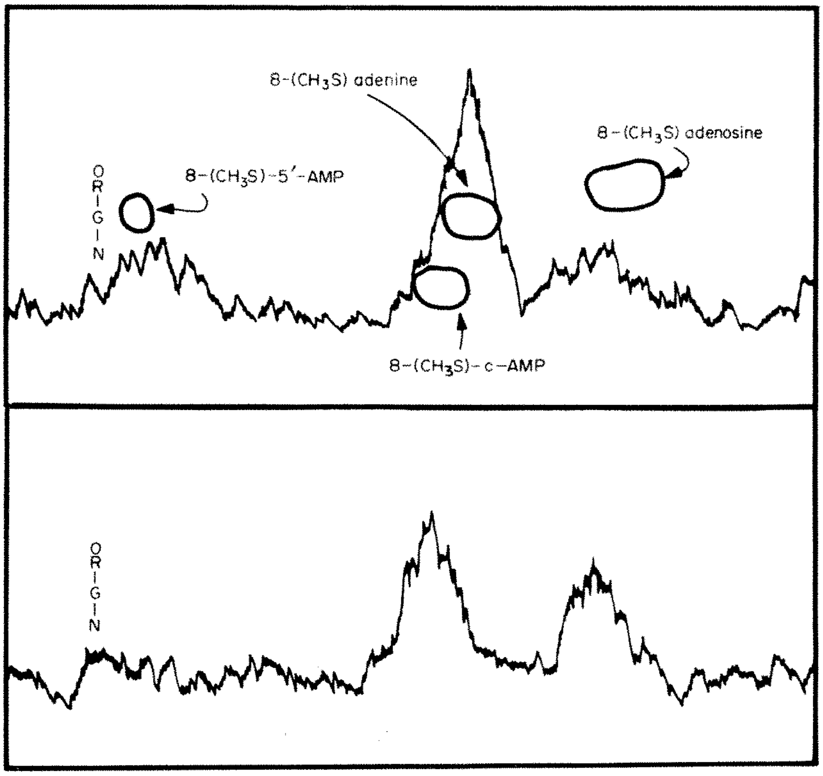

FIGURE 3. Radioscans of extracts of the intact skin of Dogs Q2-267 (upper) and Q2-259 (lower). The dogs were sacrificed 2 days after they had been given 2·5 mg/kg doses of 8-(methylthio)cyclic AMP-^{35}S topically. Solvent system 1 was employed. The circled areas represent the absorption of ultraviolet light at 254 nm for the reference compounds. Although the reference compounds are not shown in the lower chromatogram, the R$_f$ values were the same as in the upper chromatogram.

TABLE 4. Disposition of radioactivity after the topical application of 8-(methylthio)cyclic AMP-^{35}S to the intact skin of normal subjects

	% of dose* (intact skin)			
	Subject E.D.	Subject E.P.	Subject R.G.	Mean ± s.e.
Radioactivity recovered from the skin 12 h after dosing	20·61	17·54	20·88	19·68 ± 1·07
Radioactivity present on the occluding wrap and tape 12 h after dosing	23·94	14·82	72·28	37·01 ± 17·83
Radioactivity excreted in urine during 96 h	0·00	0·00	0·00	0·00
Radioactivity excreted in faeces during 96 h	0·00	0·00	0·00	0·00
Total radioactivity recovered	44·55	32·36	93·16	56·69 ± 18·57

* Each subject was given a single 25 mg topical dose.

activity in their plasma nor did they excrete any radioactivity in the urine and faeces in 4 days (Table 4). Table 5 shows the data for the subjects whose skin had been stripped prior to the application of drug. Two of these subjects (W.P. and R.B.) exhibited significant concentrations of radioactivity in the plasma and also in urine. In particular, the plasma concentrations of radioactivity for subject R.B. were the most significant, as shown in Fig. 4. For this subject, the half-time for the elimination of radioactivity from the plasma was about 0·5 h during the interval of 0·25 to 2 h after dosing, and about 11 h during the interval of 4 to 12 h after dosing. Measurements of the binding of radioactivity to the plasma proteins were made at several times during the first 2 h after dosing of subjects W.P. and R.B. An average of 48·1 ± 4·7% (± s.e.) was bound to the plasma proteins. As had been found for the dogs, substantial amounts of radioactivity were present on the occluding wrap and tape 12 h after dosing,

TABLE 5. Disposition of radioactivity after the topical administration of 8-(methylthio)cyclic AMP-^{35}S to the stripped skin of normal subjects

	% of dose* (stripped skin)			
	Subject R.B.	Subject H.L.	Subject W.P.	Mean ± s.e.
Radioactivity recovered from the skin 12 h after dosing	5·22	23·40	6·33	11·65 ± 5·88
Radioactivity present on the occluding wrap and tape 12 h after dosing	64·59	38·51	83·05	62·05 ± 12·92
Radioactivity excreted in urine during 96 h	22·94	0·48	8·18	10·53 ± 6·59
Radioactivity excreted in faeces during 96 h	0·40	0·00	0·08	0·16 ± 0·12
Total radioactivity recovered	93·15	62·39	97·64	84·39 ± 11·08

* Each subject was given a single 25 mg topical dose.

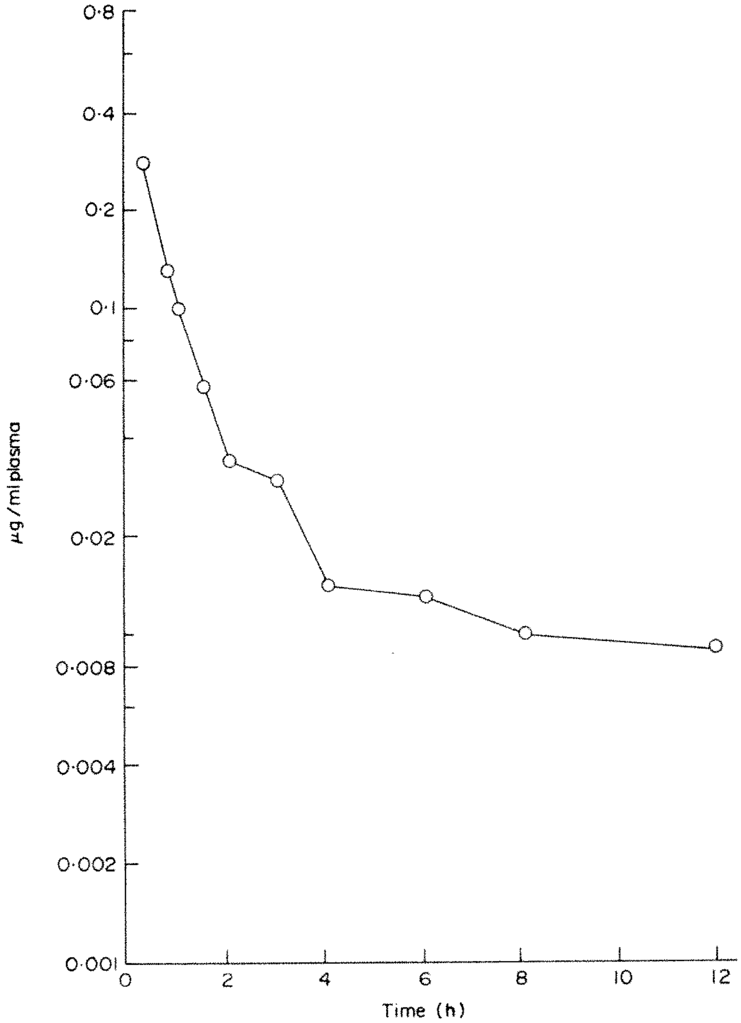

FIGURE 4. Concentrations of radioactivity in the plasma of human subject R.B. after the topical administration of a 25-mg dose of 8-(methylthio)cyclic AMP-^{35}S to stripped skin.

indicating that a large portion of the dose was unavailable for absorption. Indeed, the amounts of radioactivity excreted in the urine of subjects W.P. and R.B. were substantial during the first 2 h after dosing, and trivial thereafter. In addition, the rapid decline in concentrations of radioactivity in the plasma of subjects W.P. and R.B. shortly after dosing supports the notion that the drug was not being readily absorbed for more than a small fraction of the 12 h period of application. Thus, the conditions for the application and localization of 8-(methylthio)cyclic AMP-^{35}S do not appear to be ideal for optimizing the bioavailability of the drug. The total recovery of radioactivity was almost quantitative for three of the six subjects, one from the group with normal skin and two from the group with stripped skin. It is not known why the recovery of radioactivity was very poor for subjects E.D. and E.P.

The 0–2 h urine samples of subjects W.P. and R.B. contained sufficient radioactivity to allow for their chromatography. The results were comparable for both subjects. The chromatograms of the

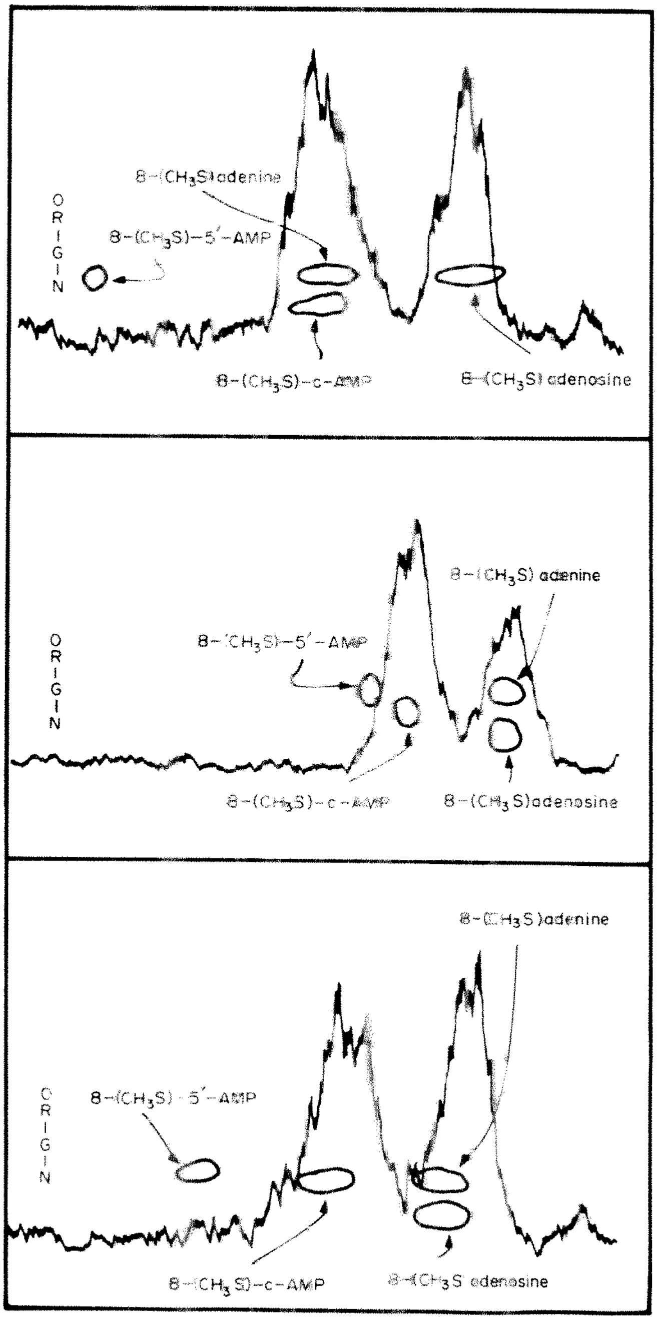

FIGURE 5. Radioscans of the urine excreted by human subject R.B. during the first 2 h after the topical administration of a 25-mg dose of 8-(methylthio)cyclic AMP-^{35}S to stripped skin. Solvent systems 1 (upper), 2 (middle), and 3 (lower) were employed. The circled areas represent the absorption of ultraviolet light at 254 nm for the reference compounds.

urine of subject R.B., developed in three solvent systems, are shown in Fig. 5. Two principal radioactive components were found in each of the three solvent systems. One of these, corresponding in all three solvent systems to unchanged 8-(methylthio)cyclic AMP-^{35}S, amounted to 54%. The other component, corresponding in all three solvent systems to 8-(methylthio)adenosine, amounted to 41%. It is worth emphasizing that solvent system 3 separates 8-(methylthio)cyclic AMP from 8-(methylthio) adenine, and solvent system 1 separates 8-(methylthio)adenine from 8-(methylthio)adenosine. To provide further evidence for the identity of the parent drug in urine, authentic 8-(methylthio)cyclic AMP-^{35}S was added to the urine sample of subject R.B. before it was chromatographed. Subsequent chromatography of this sample of urine in all three solvent systems indicated that the R_f value of the exogenous drug was the same as that of the parent drug originally present in the urine; when the chromatogram was scanned, the exogenous radioactivity was found to have become additive with that originally present in the urine.

None of the subjects showed evidence of either irritation to the skin or changes in any of the laboratory tests reported. Especially significant was the absence of any definitive changes in blood glucose or insulin for subject R.B., where 23% of the 8-(methylthio)cyclic AMP-^{35}S had been absorbed.

DISCUSSION

The results of the present studies show that small amounts of 8-(methylthio)cyclic AMP-^{35}S penetrate the intact skin of dogs, but none of it penetrates the intact skin of humans. By contrast, substantial amounts of drug penetrate the abraded skin of dogs or the stripped skin of humans, even though the drug formulation does not appear to optimize its bioavailability. The major metabolite found in the urine of humans after topical dosing, in the urine of dogs after oral or intravenous dosing (Dreyfuss *et al.*, 1975), and in the skin of dogs after topical dosing was 8-(methylthio)adenosine. All urine samples collected from dogs and humans shortly after topical dosing contained considerable amounts of unchanged 8-(methylthio)cyclic AMP-^{35}S.

It is, perhaps, not surprising that 8-(methylthio)adenosine is found as a metabolite in the skin of dogs since the skin of other species is known to contain enzymes involved in the synthesis and degradation of cyclic AMP. For example, a deficiency of adenyl cyclase in the skin of psoriatic patients has been reported (Wright *et al.*, 1973; Härkönen *et al.*, 1974), as well as less activity of cyclic nucleotide phosphodiesterase in psoriatic plaques than in healthy epidermis (Härkönen *et al.*, 1974). Cyclic nucleotide phosphodiesterase has also been detected in the skin of mice (Mier & Urselmann, 1970) and of rats (King *et al.*, 1974).

ACKNOWLEDGMENTS

We thank Mr Peter Egli for the preparation of 8-(methylthio)cyclic AMP-^{35}S, Mr Carmen Mondi and Mr John J.Ross, Jr. for their excellent technical assistance, Dr C.Parekh for the determination of glucose in plasma, Dr D.Harris for the determination of insulin in the plasma of dogs, Dr G. Faloona for the determination of glucagon and insulin in the plasma of humans, and Mr S.Meloni for the preparation of the illustrations.

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Lysosomal hydrolases of the epidermis

2. ESTER HYDROLASES

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Accepted for publication 28 October 1974

SUMMARY

Five distinct ester hydrolases (EC 3.1) have been characterized in guinea-pig epidermis. These are carboxylic esterase, acid phosphatase, pyrophosphatase, and arylsulphatases A and B. Their properties are consistent with those of lysosomal enzymes.

The ester hydrolases (EC 3.1) include enzymes capable of degrading carboxylic esters (EC 3.1.1), phosphate esters (EC 3.1.3 and 3.1.4) and sulphate esters (EC 3.1.6). As in the case of the glycosidases (Mier & van den Hurk, 1975), few quantitative studies have so far been published regarding epidermis. Even acid phosphatase, which is the best-known member of the ester hydrolases, has received scant attention from biochemists despite its routine use by histologists as a marker for lysosomes.

We have now characterized and quantified representative members of this group in guinea-pig epidermis, and our findings are presented in this communication.

MATERIALS AND METHODS

Fluorogenic substrates used in this investigation are listed in Table 1. All other materials were precisely as described in our previous report (Mier & van den Hurk, 1975).

Assay procedures

Preliminary work again indicated that the use of 4-methyl umbelliferyl (4-MU) derivatives provided considerably greater sensitivity than alternative fluorimetric or colorimetric techniques. Individual enzymes assayed, together with details of substrate concentration, epidermal concentration and pH employed are listed in Table 1.

The method of assay was in general identical to that described previously (Mier & van den Hurk, 1975). The exception was in the case of the carboxylic ester hydrolases; because of the instability of the 4-MU carboxylic acid esters to alkali, the reaction was terminated by the addition of phosphate buffer (0·2 mol/l, pH 7·4) rather than the carbonate–bicarbonate buffer normally employed, and the fluorescence ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 450$ nm) was read within 30 s.

TABLE I. Substrates and reaction conditions used for the assay of individual ester hydrolases

EC number	Enzyme	Substrate	Source	Reaction conditions: Concentration of buffered substrate (mmol/l)	Reaction conditions: Concentration of epidermal extract (mg/ml)	Reaction conditions: pH
3.1.1.–	Esterase	4-MU acetate 4-MU butyrate 4-MU nonanoate 4-MU palmitate	Koch–Light	0·5	5	4·3
3.1.3.2	Acid phosphatase	4-MU phosphate	Sigma	0·2	0·5	3·6
3.1.4.1 3.6.1.–	Pyrophosphatase	4-MU pyrophosphate	Koch–Light	0·2	5	4·8
3.1.6.1	Arylsulphatase A	4-MU sulphate	Sigma	0·5	10	4·6
3.1.6.1	Arylsulphatase B	4-MU sulphate	Sigma	2·0	10	5·9

Effects of reaction variables

Experiments to determine the effects of certain reaction variables were carried out in the manner described in our previous communication (Mier & van den Hurk, 1975); in all cases the precise reaction conditions, other than the variable being investigated, were as shown in Table 1.

The influence of pH upon the rate of hydrolysis was determined for all substrates listed in Table 1. Progress curves, the effect of epidermal concentration and the effect of substrate concentration were studied using 4-MU palmitate, phosphate, pyrophosphate and sulphate; in the last case, independent kinetic studies were carried out at pH 4·6 and pH 5·9.

Normal values

The activities of palmitate esterase, acid phosphatase, pyrophosphatase and arylsulphatase A and B were determined in fresh epidermal extracts prepared from four different animals.

RESULTS

Progress curves

These are illustrated in Fig. 1. It is seen that acid phosphatase and arylsulphatase showed a linear reaction rate for about 1 h, and palmitate esterase and pyrophosphatase for at least 2 h under the conditions described.

Effect of epidermal concentration

The reaction rate was proportional to the concentration of the epidermal extract up to 2 mg/ml for acid phosphatase and up to at least 10 mg/ml in the case of the remaining enzymes.

Effect of pH

The rates of hydrolysis of each of the four carboxylic acid esters at pH values in the range 3·8–5·9 are shown in Fig. 2(a). Since there was no indication of a discrete enzyme active against the short-chain esters (C_2 to C_9) at acid pH, these substrates were not investigated further. In the case of the palmitate, however, a broad optimum was observed in the range pH 4·0–5·5.

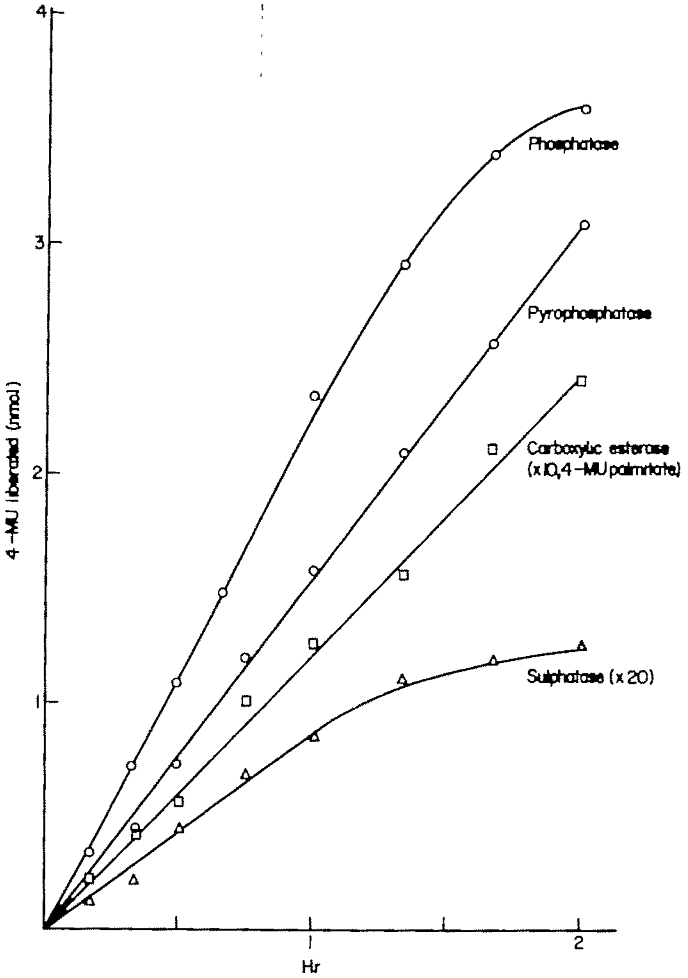

FIGURE 1. Progress curves showing the release of 4-MU at periods up to 2 h. The pH of the incubation mixture for sulphatase was 5·4; otherwise all conditions were as in Table 1.

Acid phosphatase and pyrophosphatase both displayed single peaks (Fig. 2b), and sulphatase two peaks of activity (Fig. 2c) in the range examined. The pH optima of the individual enzymes are listed in Table 2.

Enzyme kinetics

Lineweaver and Burk plots for carboxylic esterase (Fig. 3a), acid phosphatase (Fig. 3b) and pyrophosphatase (Fig. 3c) were linear over the range of substrate concentrations employed. Michaelis–Menton constants obtained by extrapolation are listed in Table 2.

The kinetics of hydrolysis of 4-MU sulphate were examined independently at pH 4·6 and pH 5·9 (corresponding to the peaks shown in Fig. 2c). The Lineweaver and Burk plots were in both cases biphasic (Fig. 3d).

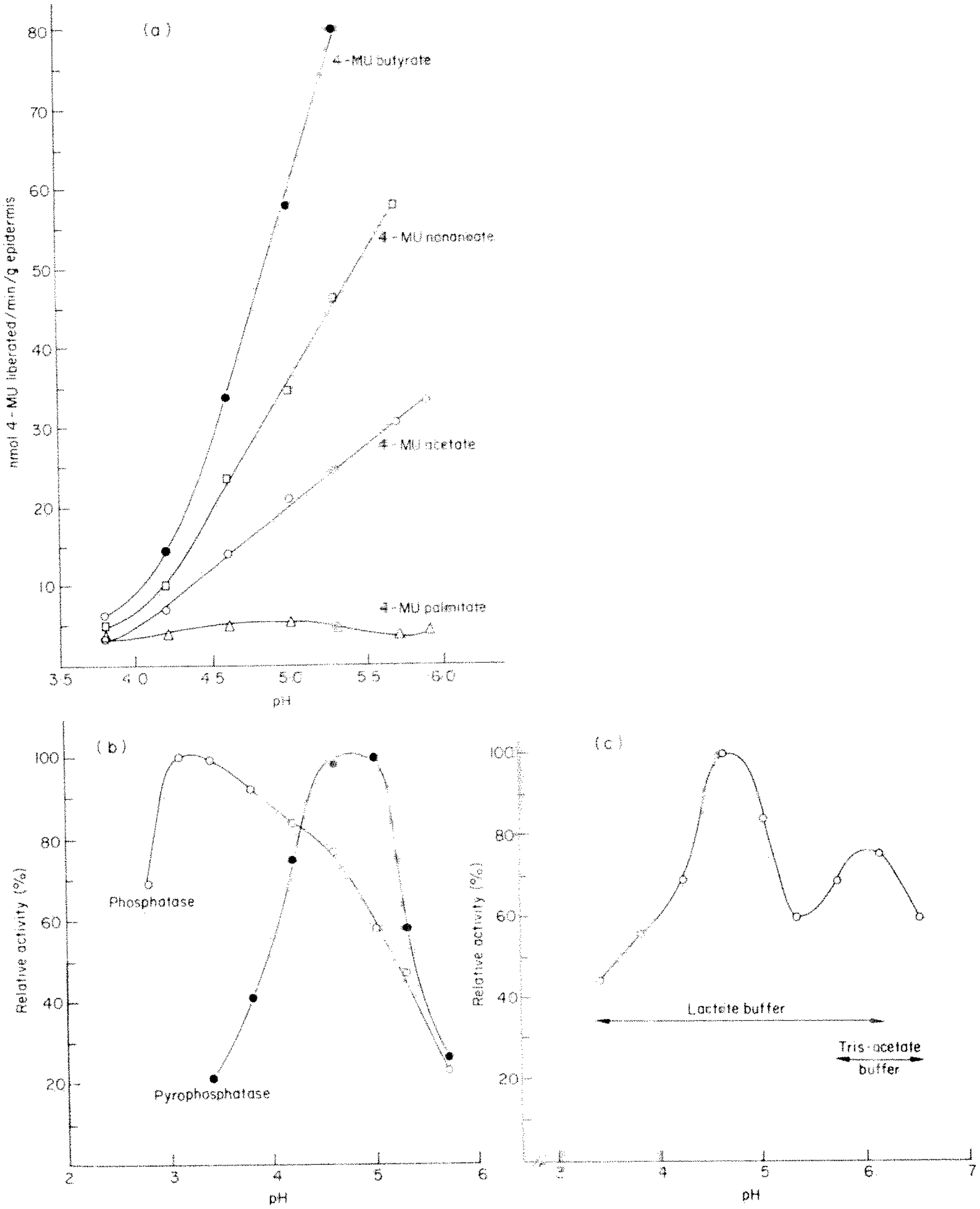

FIGURE 2. The effects of pH on the enzymatic activities. In the case of the carboxylic esterase (a), absolute levels of activity have been used in order to provide a direct comparison between the various substrates; for the remaining enzymes (b and c) the activities are shown as a percentage of maximum. The concentration of 4-MU sulphate in (c) was 0·5 mmol/l (final), otherwise all conditions were as in Table 1.

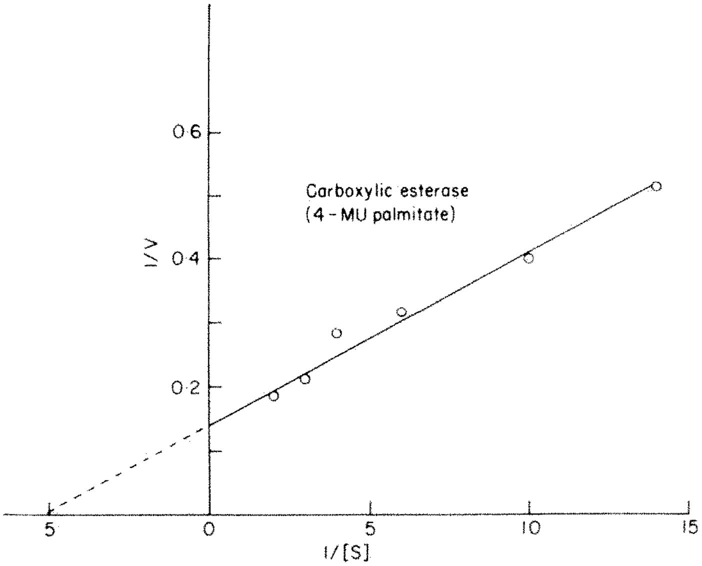

FIG. 3a.

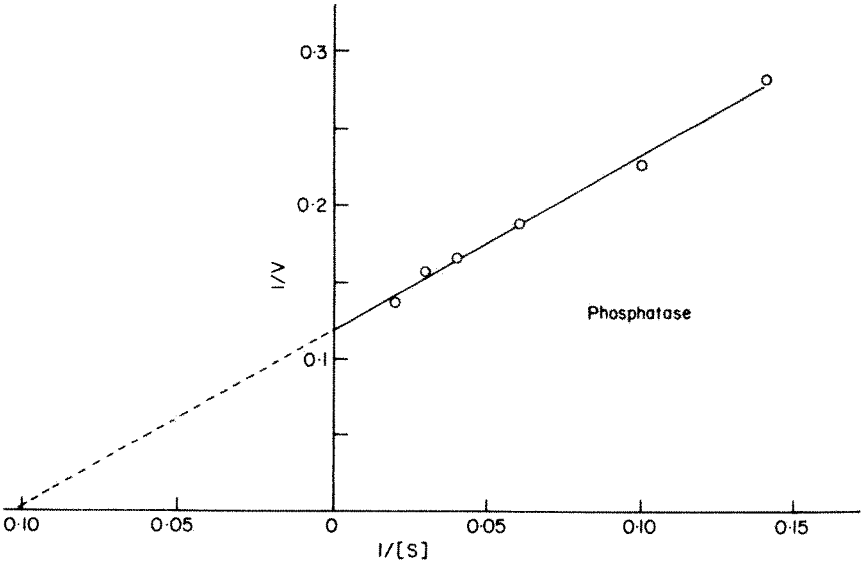

FIG. 3b.

FIGURE 3. Lineweaver and Burk plots of the epidermal ester hydrolases. Units are as follows: (a) V = nmol/min/g, S = mmol/l, (b) V = μmol/min/g, S = μmol/l, (c) V = μmol/min/g, S = mmol/l, (d) V = nmol/min/g, S = mmol/l.

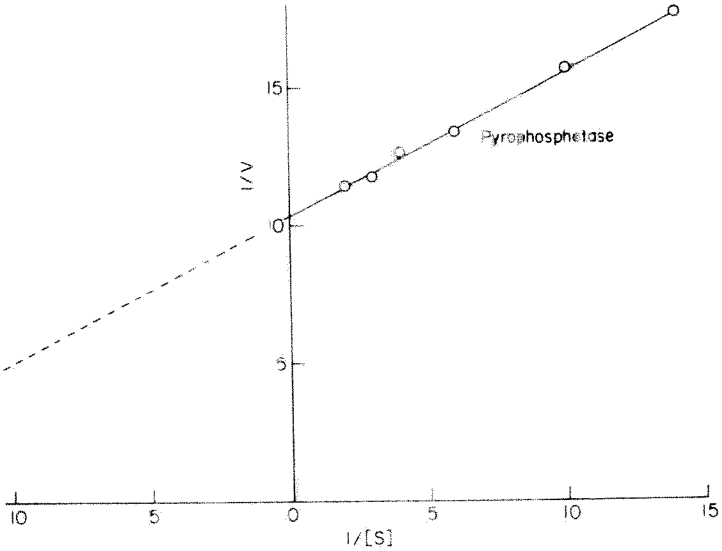
15
10
5
1/V
Pyrophosphetase
10
5
0
5
10
15
1/[S]

FIG. 3c.

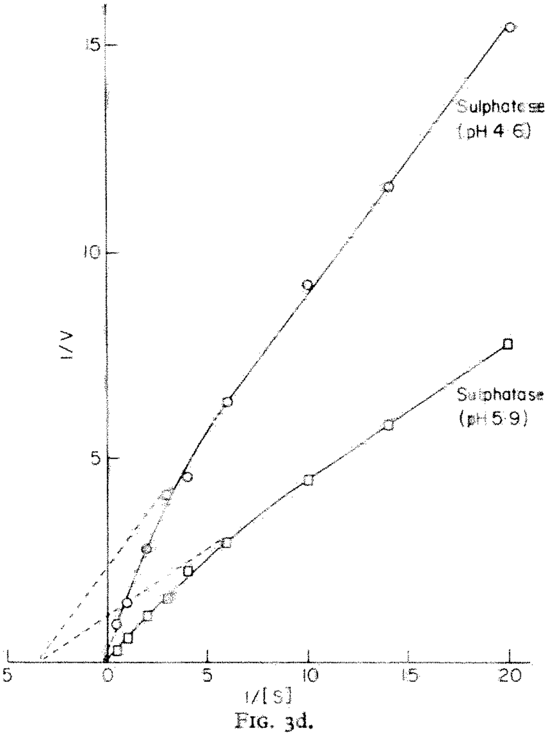
15
10
5
1/V
Sulphatase
(pH 4·6)
Sulphatase
(pH 5·9)
5
0
5
10
15
20
1/[S]

FIG. 3d.

TABLE 2. Properties of the ester hydrolases of guinea-pig epidermis

Enzyme	pH optimum	Michaelis–Menton constant (mol/l)
Esterase (4-MU palmitate)	4·0–5·5	$1\cdot9\times10^{-4}$
Acid phosphatase	3·2	$1\cdot0\times10^{-5}$
Pyrophosphatase	4·8	$5\cdot1\times10^{-5}$
Arylsulphatase A	4·6	$2\cdot8\times10^{-4}$
Arylsulphatase B	6·0	about 5×10^{-3}

TABLE 3. Activities of epidermal esterases in preparations from 4 different guinea-pigs. All values are in nmol (10^{-9} mol) of 4-MU liberated per min per g fresh weight of epidermis

Enzyme	Animal no.				Mean
	1	2	3	4	
Esterase (4-MU palmitate)	3·8	3·9	6·5	5·9	5·0
Acid phosphatase	490	950	1150	1330	930
Pyrophosphatase	44	68	64	98	69
Arylsulphatase A	0·39	0·44	0·67	0·44	0·49
Arylsulphatase B	1·4	1·3	1·9	1·2	1·5

Normal values

The activities of each enzyme determined in a series of fresh epidermal extracts are shown in Table 3.

DISCUSSION

Our results demonstrate the presence of at least five distinct ester hydrolases active at acid pH in guinea-pig epidermis. Because of the heterogeneity of this group, it is more convenient to discuss the various enzymes separately.

Carboxylic esterases

The failure to observe any discrete peak of activity at acid pH against C_2, C_4 or C_9 esters is undoubtedly the result of interference from the high level of microsomal (neutral) esterase present in our preparation. Mahadevan & Tappel (1968a) showed the lysosomal esterase of liver or kidney to attack esters in the range C_8–C_{18} at roughly similar rates; the neutral esterase, on the other hand, has maximal activity against short or medium-chain esters but negligible effect on long-chain substrates. The latter observation has recently been confirmed for epidermis (Mier & van den Hurk, 1974).

The properties of the epidermal carboxylic acid esterase (using 4-MU palmitate as substrate) are reasonably similar to those of isolated lysosomal preparations; Mahadevan & Tappel (1968a), for example, reported a pH optimum of 3·6–4·0 and a K_m of 1·0 mmol/l using p-nitrophenyl myristate as substrate. It is not yet clear whether the lysosomal esterase is distinct from the lysosomal lipase (EC 3.1.1.3) described by Mahadevan & Tappel (1968b).

Acid phosphatase

This is by far the most active of the lysosomal esterases in epidermis, as it is in other tissues. Our value of 930 nmol/min/g fresh weight may be compared with figures of 13·15 nmol/min/mg protein (Clausen, Melchior & Paerregaard, 1972) and 7·71 nmol/min/mg protein (Ockerman, 1968) obtained using whole human skin. The properties of the epidermal enzyme seem quite similar to those reported for isolated lysosomal preparations (reviewed by Barrett, 1972).

Pyrophosphatase

Although this enzyme was originally given a separate classification (EC 3.6.1.–), it is probably identical to phosphodiesterase IV (EC 3.1.4.1), which hydrolyses nucleotides such as FAD and ATP (Brightwell & Tappel, 1968). The activity of this latter enzyme has been measured in human epidermis by de Bersaques (1968); using di(p-nitrophenyl) phosphate and p-nitrophenyl thymidine phosphate as substrates, he obtained values in the range 10–20 μmol/h/g dry weight, figures remarkably similar to our own.

Sulphatase

Both the pH curve (Fig. 2c) and the Lineweaver & Burk plots (Fig. 3d) make it clear that epidermis contains two distinct sulphatases. The actual pH optima and the ratio of the Michaelis–Menton constants leave little doubt that these are arylsulphatases A and B (reviewed by Barrett, 1972).

Although there is histochemical evidence that sulphatase activity is associated with lysosomes in the epidermis of mouse (Rowden, 1968) and man (Olsen, Nordquist & Everett, 1968), the only previous quantification of this enzyme in skin is that described by Goldberg *et al.* (1971). These workers reported a value of 1·3 nmol/min/g fresh weight for whole human skin (p-nitrophenyl sulphate, pH 4·5).

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British Journal of Dermatology (1975) **93**, 399.

Median rhomboid glossitis

CANDIDIASIS AND NOT A DEVELOPMENTAL ANOMALY*

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Accepted for publication 23 December 1974

SUMMARY

Infection by Candida Albicans was found in all biopsies taken from ten patients presenting with midline lesions of the tongue which clinically presented as median rhomboid glossitis.

The subject of this paper concerns a relatively uncommon anomaly of the tongue to which the rather grandiloquent title of 'Glossite losangique médiane de la face dorsale de la langue' was first given by Brocq & Pautrier (1914). In common with many other conditions where the diagnosis can be made confidently on the clinical features alone, biopsies are rarely performed and there is often only speculation regarding the underlying pathology. On *a priori* evidence Martin & Howe (1938) propounded the very plausible theory that median rhomboid glossitis results from the persistence of the tuberculum impar on the surface of the tongue.

It was the striking appearance of the lesion that not unnaturally provoked cancerophobia in the mind of the patient and resulted in advice being sought. Just occasionally there was discomfort, generally in the form of a burning sensation, and if it persisted the lesion was excised. The diagnosis of median rhomboid glossitis has not been a comfortable one and the purpose of this study is to provide support for those who believe that the condition is inflammatory and not developmental and is in fact a pattern of chronic hyperplastic candidiasis.

Sammett in 1939, in the most unlikely of all journals for this subject, namely the *Radiology*, set out six diagnostic clinical features:

(a) The characteristic location in the median raphe anterior to the circumvallate papillae.
(b) Its roughly rhomboidal pattern.
(c) Its rosy colour.
(d) Its smooth common nodular or fissured surface devoid of papillae.
(e) Its slight induration.
(f) Its asymptomatic character and chance discovery.

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* Part of the Presidential Address to the Section of Odontology of the Royal Society of Medicine, 28 October 1974.

For some time, I have been puzzled by the many variations that can occur within these six diagnostic features for certainly, though all the lesions are generally in the median raphe, they are not always just anterior to the circumvallate papillae and may appear well forward in the midline of the dorsum of the tongue. The lesions are not always asymptomatic and increasingly they are the cause of discomfort and particularly a burning sensation. Certainly they are always without papillae but may vary in colour from the bright red, to red flecked with white, to frankly white patches within the well demarcated zone (Figs 1 and 2). To the best of my knowledge the first serious suggestion that median rhomboid glossitis was not a developmental anomaly appeared in a paper by R.A.Baughman in 1971 and he favoured the lesion to be either inflammatory, infective or degenerative in nature. In his paper he reviewed the literature and noted that no case had ever been reported in a child, and he himself examined the tongues of 10,010 children from kindergarten to the 12th grade at school and did not find a single example. He also could not find any evidence of median rhomboid glossitis in the tongues of 184 cadavers. He then examined the histology of seven cases labelled as median rhomboid glossitis in the files. All were Caucasians, six were female, one male and all aged between 39 and 62 years. His study of the histological features revealed similar findings to three cases described by the writer in 1962. There is parakeratosis of the stratified squamous epithelium devoid of papillae. There are deep branching epithelial ridges described as test-tube like and the subepithelial foci of lymphocytes are the most striking feature. There is no atypia of the epithelium and the supra-muscular sclerosis is probably normal to the median raphe of the tongue. I think that the first reference

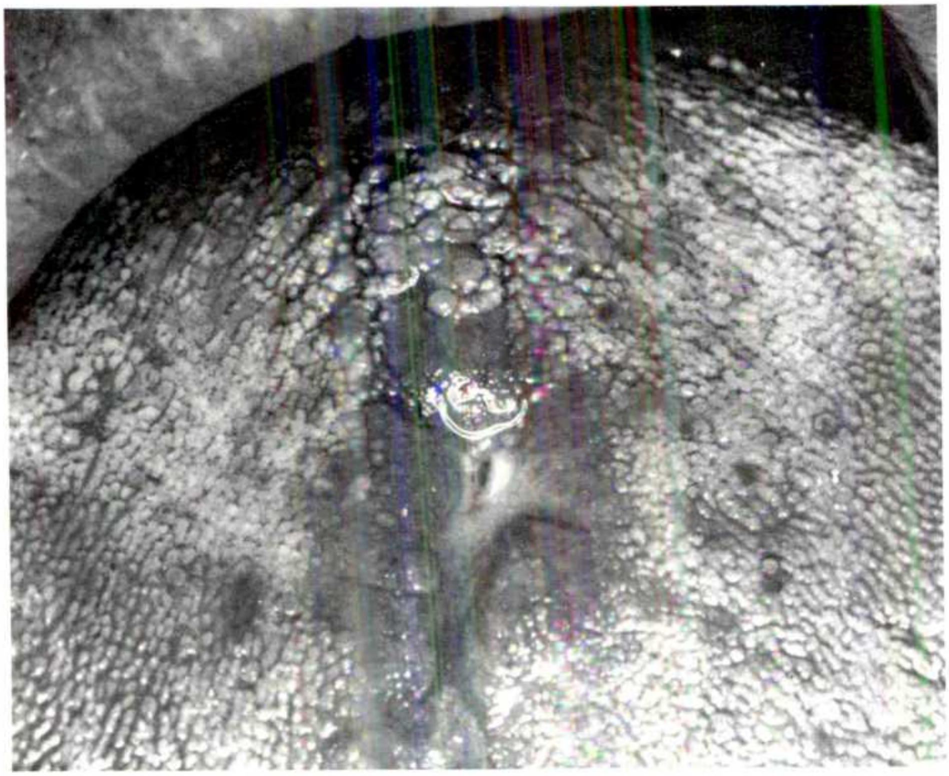

FIGURE 1. Male aged 60 years. Chronic candidiasis with midline fissure of dorsum of tongue.

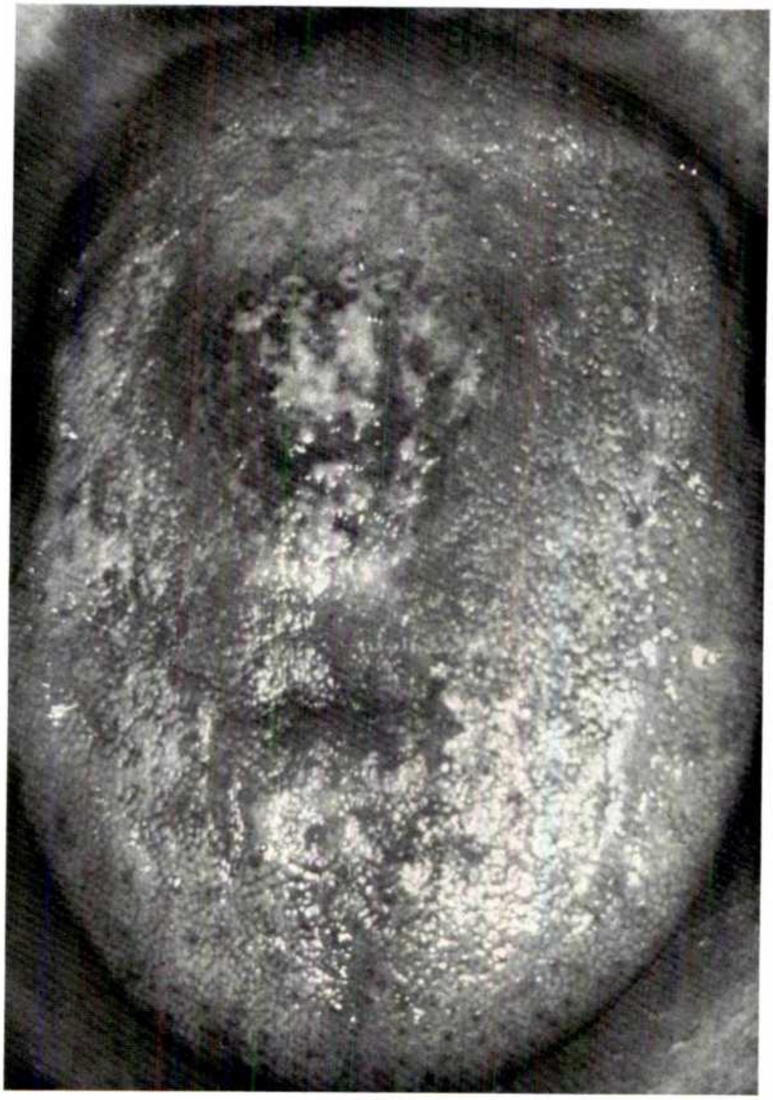

FIGURE 2. Female aged 35 years with acute pseudo-membranous candidiasis superimposed on chronic candidiasis of midline of dorsum of tongue.

to the possibility that this is a candidal lesion is made by J.J.Pindborg in the *Textbook of Dermatology* (2nd edn) edited by Rook *et al.* (1972).

Patients

Ten patients presented with chronic median glossitis. Six were female, four male, and their ages ranged from 30 to 63 years, with four in the fourth decade, three in the fifth, and three in the seventh. All the patients complained of discomfort, mainly a burning sensation and these symptoms were present for up to a year before examination. The lesions ranged from a position just anterior to the circumvallate papillae to the junction of the anterior and the middle third of the tongue, but all were in the midline. They varied from being smooth to grossly nodular and fissured. There was loss of suppleness within them all and with a degree of induration in some. There were varying degrees of a superimposed acute candidiasis so that the surface would sometimes appear thrushlike in part or in whole. One of the patients gave a history of having had warts removed from the dorsum of the tongue 3 years previously and presented with four or five papules about 3 mm in diameter in the middle of the dorsum of the tongue.

As the condition was recognized to be probably a form of chronic candidiasis, antifungal therapy was prescribed for these patients but with very limited success.

Histopathology

Candidal hyphae were found within the superficial layers of the parakeratotic epithelium in all cases (Fig. 3). Where there was a superimposed acute candidiasis there was a plaque of epithelial cells and chronic inflammatory cells invaded by the candida albicans superimposed upon the parakeratotic epithelium (Fig. 4). Test-tube like deep epithelial ridges, some of which branched, were also very striking features. Furthermore, there were foci of lymphocytes closely related to the

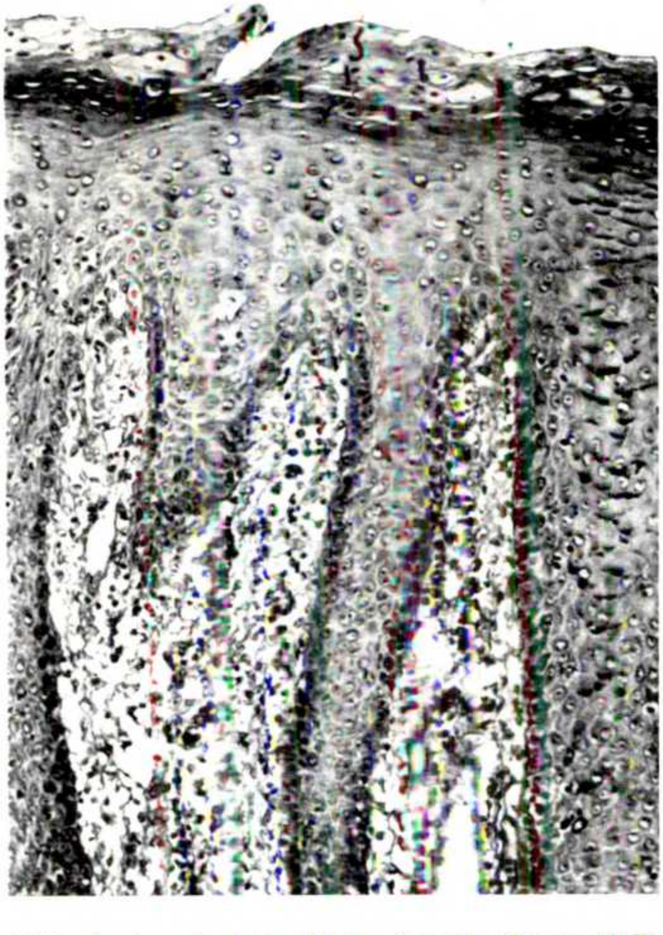

FIGURE 3. Candidal hyphae in parakeratotic epithelium from lesion illustrated in Fig. 1 (PAS × 40).

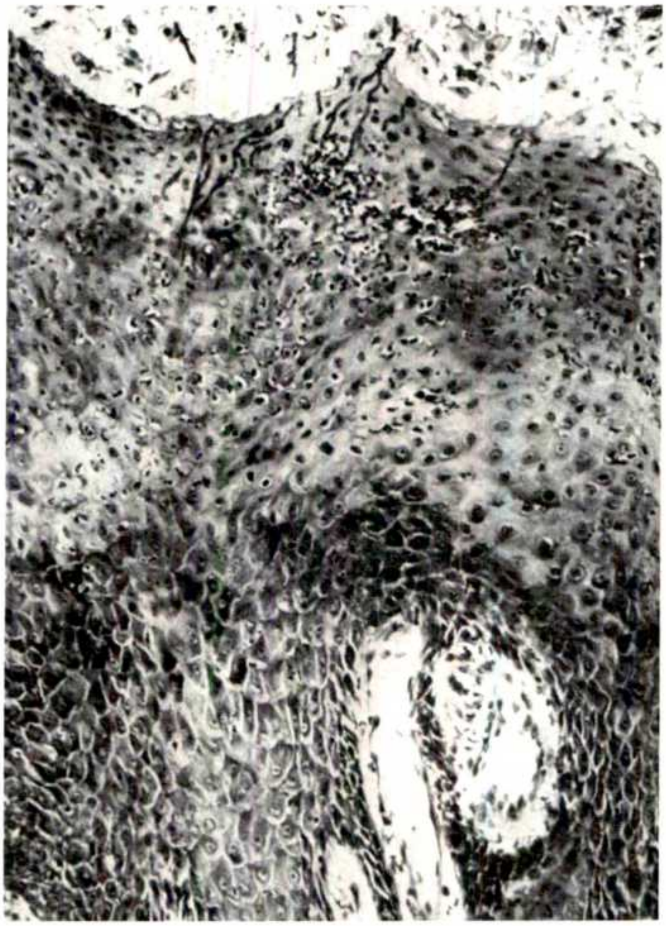

FIGURE 4. Candidal hyphae within the superficial plaque of epithelial cells and within the parakeratotic epithelium of lesion illustrated in Fig. 2 (PAS × 40).

epithelial ridges, particularly near the tips and there were many dilated capillaries within the corium between the epithelial ridges (Fig. 5). The degree of oedema between the superficial prickle cells varied from case to case, but in one there were micro-abscesses filled with polymorphonuclear leukocytes. The intercellular oedema and the infiltrate of lymphocytes and occasional polymorphonuclear leukocytes disturbed the arrangement of the prickle cells, but no atypia was observed.

DISCUSSION

In 1966 Cawson described the clinical and histological features of chronic oral candidiasis and made three important points; first, the presence of hyphae invading the epithelium was a positive finding that cannot easily be ignored; secondly, the infection was associated with a fairly consistent set of histological changes; and thirdly, candida was not seen to be superimposed on some other recognizable pathological process. As in his cases, so in those under discussion, the tendency of the superficial epithelial layers to separate once candidal infection was established meant that the number of hyphae observed in the PAS sections varied considerably from just a few to a heavy invasion.

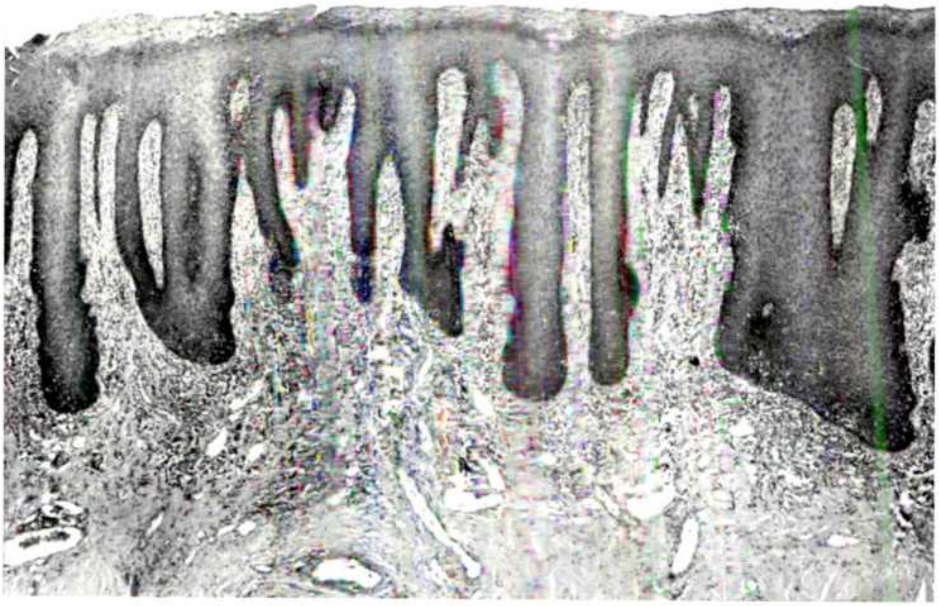

FIGURE 5. Parakeratotic and acanthotic epithelium with 'test-tube like' deep epithelial ridges and foci of lymphocytes within the corium from the lesion illustrated in Figs 1 and 3.

The site of election suggests that the foramen caecum and perhaps the depression of the midline raphe of the tongue favour the localization of candidal infection. From these sites it then spreads laterally and is characterized by its chronicity. There is little doubt that the loss of the papillae is permanent and that the histological changes induced by the candidal hyphae are irreversible and permanent. Otherwise, how could the histological appearances be described in such a uniform manner by so many different authors over the years? The slides illustrated in Figs 3 and 4 of the author's paper (1962) have been stained with PAS and candidal hyphae have been identified in the parakeratotic epithelium.

Of the greatest relevance have been the observations made by Jones & Russell (1974) in a recent paper entitled 'The histology of chronic candidal infection of the rat's tongue'. The changes they observed in the lingual epithelium were very similar to the findings just described, with the addition that some dysplasia of the epithelium was noted, together with muscle changes as seen in the regenerative phases following damage to muscle. This, the authors presumed, might eventually lead to extensive sub-epithelial fibrosis and alter the ability of the oral mucosa to respond to any potentially harmful stimulus.

No treatment is either necessary or effective in those asymptomatic cases with a smooth, nodular or fissured surface devoid of papillae. However, in those lesions with a fissured surface associated with a persistent painful glossitis, excision is indicated.

Anti-fungal therapy would appear only to correct any superimposed pseudo-membranous candidiasis, although it may well be efficacious in the treatment of the early lesions, so preventing the condition becoming permanent.

In conclusion, therefore, it is submitted that the condition that has been described as median rhomboid glossitis is chronic hyperplastic candidiasis and not a developmental anomaly.

ACKNOWLEDGMENTS

I am grateful to Mr John Wolfle and Mr Russel Hopkins for undertaking the surgical treatment of many of the patients referred to in this paper; Mr R.T.Allison for technical assistance in the preparation of the sections and photomicrographs; and to Mr B.A.Jones of the Dental Audio-visual Aids Unit for the clinical photographs.

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British Journal of Dermatology (1975) **93**, 407.

Plane xanthomata associated with multiple myeloma and hyperlipoproteinaemia

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Accepted for publication 20 January 1975

SUMMARY

A case of plane xanthomatosis associated with multiple myeloma and hyperlipoproteinaemia in a 62-year-old woman is reported. The patient had IgG type lambda paraproteinaemia and type II A hyperlipoproteinaemia.

The IgG paraprotein showed binding activity against high-density lipoproteins. It could not be demonstrated that these paraprotein–lipoprotein complexes were responsible for the hyperlipidaemia.

Microchemical analysis of pathological skin demonstrated a high content of triglycerides, similar to that in eruptive xanthomata. No correlation was found between the lipid composition of the xanthomatous skin and the serum lipid composition.

The results obtained with an *in vitro* culture method showed that the paraprotein present in the patient's serum was synthesized in the bone marrow but not in the normal or pathological skin.

Plane xanthomata, described by Lynch & Winkelmann (1966), are yellow to yellowish-brown flat patches or slightly elevated plaques. When generalized, the disease is frequently associated with multiple myeloma or other reticulo-endothelial malignancies (Beaumont *et al.*, 1965; Lynch & Winkelmann, 1966; Feiwel, 1968; Winkelmann & Welborn, 1969; Moschella, 1970). Although assigned to the group of normolipidaemic xanthomata (Altman & Winkelmann, 1962; Fleischmayer, Hyman & Weidman, 1964; Bazex, Dupré & Abravanel, 1965), occasional cases with concomitant hyperlipoproteinaemia type II or IV have been observed.

Several authors have described the occurrence in patients with multiple myeloma of a paraprotein with binding activity against high-density or low-density lipoproteins (Kayden, Franklin & Rosenberg, 1962; Beaumont *et al.*, 1965; Aubert *et al.*, 1967; Riesen, Noseda & Bütler, 1972; Kodama, Nakagawa & Tanioku, 1972). This antibody-like activity of myeloma proteins can be associated either with hyperlipidaemia or with hypolipidaemia (Riesen *et al.*, 1972).

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The patient reported here showed plane xanthomata, hyperlipoproteinaemia type II, and IgG-myeloma. In this case binding activity of the paraprotein against high-density lipoproteins was demonstrated.

History

A 62-year-old woman was admitted (February 1973) because of extensive yellow discolouration of the skin. In 1968 complaints of headaches, dizziness, and severe back pain had led to an examination which resulted in the diagnosis of multiple myeloma, demonstrated both clinically and histologically (bone marrow aspiration). The radiograms revealed multiple osteolytic lesions in the pelvis, femora and shoulder girdles and osteoporosis of the thoracic and lumbar vertebrae. She was treated with intermittent schedules of melphalan (Alkeran, 120 mg daily) and prednisone (20 mg daily). In April 1972, a yellowish discoloration appeared in the creases of her hands. Soon afterwards large areas on the trunk and limbs also became yellowish. No improvement in the cutaneous xanthomatosis occurred under the melphalan–prednisone treatment.

Dermatological findings

The large, indistinctly demarcated yellow patches on the eyelids, neck, axillae, elbow folds, groins and spreading onto surrounding skin were characteristic of plane xanthomata. The shoulders, back, and abdomen showed numerous discrete small yellow papules, 2–3 mm in diameter (Fig. 1). The creases of the palms, fingers and wrists showed the intense yellowish discolouration characteristic of xanthochromia striata palmaris.

Laboratory investigations

The erythrocyte sedimentation rate was 121 mm/h. The haemoglobin level was 13·2 g%, and the

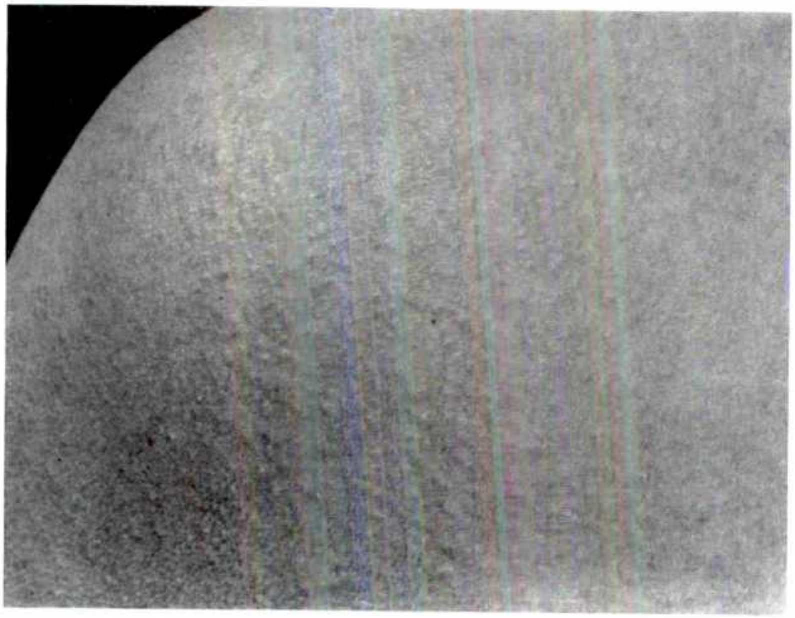

FIGURE 1. Close up of the left shoulder showing numerous small yellow papules.

white blood cell count 3000/mm^3 with a normal differential count. The liver and renal function tests, fasting blood-sugar, serum carotene, protein-bound iodine, and urinalyses were all within normal limits.

Specific investigations

(1) *Histopathological findings in the skin.* All biopsy specimens of clinically normal skin, yellowish skin, and a yellow papule showed foam cells in the corium. In the 'normal' skin these cells were relatively isolated, in the yellowish skin they occurred throughout the corium in groups as small strands between the collagen fibres (Fig. 2). The papules showed masses of foam cells occupying the subpapillary, middle and lower corium. Some cells with a double nucleus were found, but no Touton giant cells or plasma cells. In addition, there was some perivascular lymphocytic infiltration.

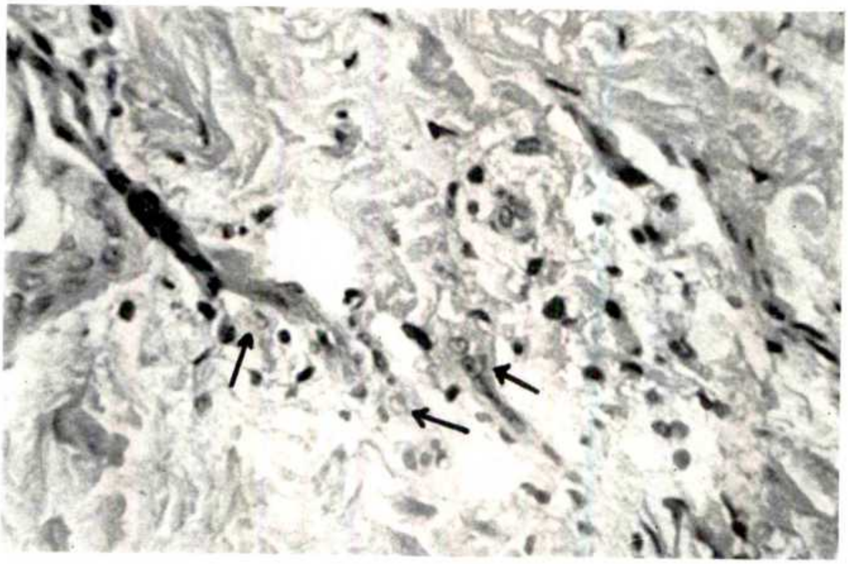

FIGURE 2. Histopathology of normal skin showing relatively isolated foam cells, disseminated through the corium.

(2) *Lipid composition of xanthomatous skin.* Samples of yellow skin and of a yellow papule were taken by punch biopsy. After as much adipose tissue as possible had been removed, the material was minced and the lipids were extracted with methanol–chloroform and purified with the Folch two-phase system.

After thin-layer chromatographic separation with silica gel, the lipid composition was determined by densitometry. Total lipids were determined gravimetrically. The results are shown in Table 1. The lipid composition of the two skin fragments was very similar; they both had a high lipid content and a high percentage of triglycerides.

(3) *Serum lipid analysis.* The total lipid concentration of the fasting serum was 1427 mg/100 ml, cholesterol 485 mg%, triglycerides 228 mg%, and phospholipids 457 mg%. The cholesterol: triglyceride ratio was 2·13.

TABLE 1. Lipid composition of xanthomata

	Total lipids (mg/g dry tissue)	% of total lipids				
		Cholesterol esters	Free cholesterol	Triglycerides	Free fatty acids	Phospholipids
Yellow skin	268	37	6	43	2	12
Papule	173	30	10	39	4	17
Eruptive xanthomata*	160–350	20–68	6–16	4–42	1–17	11–27

* According to Baes *et al.* (1968).

Compared with the normal values found in the Netherlands the cholesterol level was markedly elevated; the triglycerides and phospholipids were only slightly above normal limits.

Serum lipoproteins. Preparative separation of the serum lipoproteins was done by a combination of ultracentrifugation and precipitation. The results are given in Table 2.

The low-density lipoprotein (LDL) concentration was increased, the very-low-density (VLDL) and high-density lipoproteins (HDL) were normal. This lipoprotein pattern is typical for hyper-β-lipoproteinaemia, i.e. type IIA following the WHO classification (Beaumont *et al.*, 1970).

TABLE 2. Serum lipoprotein levels

Lipoproteins	Low density	Very low density	High density	Chylomicrons
mg %*	1042 (220–506)	86 (13–120)	285 (128–307)	14 (6–19)
weight %	73	6	20	1

* Figures in parentheses are normal range, taking patients' age into consideration, according to Polano *et al.* (1969).

TABLE 3. Lipid analysis of the lipoprotein fractions in serum

	Total serum (mg %)	Lipoprotein composition (weight %)		
		Low density	Very low density	High density
Free cholesterol	157	11	9	6
Cholesterol esters	557	43	24	32
Triglycerides	228	17	47	8
Phospholipids	457	29	17	47
Free fatty acids	28	0	3	7
Total lipids	1427			

Lipoprotein lipids. The lipid analysis of the various lipoprotein fractions was performed by quantitative thin-layer chromatography. The results are shown in Table 3. The composition of the lipids in each lipoprotein fraction was the same as that found in the primary familial type II hyperlipoproteinaemia by van Gent (1972). The cholesterol or triglyceride levels were not higher than normal. The cholesterol: triglyceride ratio in the VLDL was 0·48.

Paper electrophoresis of the serum, performed according to Lees & Hatch (1963), showed an intensely stained β-lipoprotein band. The pre-β- and α-lipoprotein bands were normal.

Electrophoresis of the isolated lipoprotein fractions showed that all of the VLDL migrated to the pre-β position. Thus, no β-VLDL ('floating β') was detected.

Family investigation. The patient has seven adult children. On examination, they all proved to be normolipidaemic: plasma lipid and lipoprotein composition were normal, as was the lipoprotein paper-electrophoresis pattern.

(4) *Immunological investigations: blood serum.* The total serum protein level was 9·67 g/100 ml, albumin 3·85 g%, α_1-globulin 0·13 g%, α_2-globulin 0·95 g%, β-globulin 1·18 g%, γ-globulin 3·69 g%. Agar electrophoresis showed an M component in the β region. Immunoelectrophoretic analysis using class- and type-specific antisera showed this paraprotein to be IgG type lambda. The quantitative analysis of the serum immunoglobulins by the Mancini technique gave a low IgA value, 38 IU/100 ml (normal 90–110) and a slightly decreased IgM value, 81 IU/100 ml (normal 110–150). The serum contained no cryoglobulins and the complement content was normal; the direct Coombs' test was negative; antinuclear antibody was dubiously positive. Bence-Jones protein was not detected in the urine.

Bone marrow. Morphologically, the marrow was hypercellular with an appreciable proportion of myeloma cells. Immunofluorescence investigation of a fixed bone marrow sample (Miss H.R.E.Schuit, Institute for Experimental Gerontology, TNO, Rijswijk) showed an increased percentage of IgG-positive cells, 98% (normal 51%). Of the remaining cells 1% were IgA-positive (normal 37%) and 1% IgM-positive (normal 12%); there were no IgD- or IgE-positive cells (Table 4). The kappa: lambda ratio of all immunoglobulin-containing cells was 0·04 (normal 2·2).

TABLE 4. Results of analysis of immunoglobulins in serum and plasma cells of bone marrow

	IgG	IgA	IgM	IgD	K:λ ratio
Immunoglobulin contents of serum* (IU/100 ml)		38 (90–110)	81 (110–150)		
Immunoglobulin-containing plasma cells in bone marrow† (rel. %)	98 (51)	1 (37)	1 (12)	0 (4)	0·04 (2·2)

* Determined according to the Mancini technique. Normal range in parentheses.

† Direct immunofluorescence technique. Normal values in parentheses (Hijmans *et al.*, 1971).

Skin. Direct immunofluorescence examination of sections of normal skin, yellow skin, and yellow papules stained with FITC labelled antihuman serum, anti-IgG, anti-IgA, and anti-IgM revealed no immunoglobulin deposits and no immunoglobulin-containing cells.

Immunoglobulin synthesis in vitro (Dr R.F.M.Lai-A-Fat, Department of Dermatology, University Hospital, Leiden). For the assay of immunoglobulin synthesis *in vitro*, the technique described by Hochwald, Thorbecke & Asofsky (1961) was applied: bone marrow flocks and minced skin tissue were incubated in a modified Eagle's medium containing ^{14}C-lysine (310 μCi/mmol) and ^{14}C-isoleucine (312 μCi/mmol) with high specific activity.

The immunoglobulins synthesized *de novo* were detected and identified by autoradiography of the immunoelectrophoretic pattern of the culture fluid. The results showed that the bone marrow synthesized an IgG type lambda paraprotein. No synthesis of immunoglobulins was found in the cultures of normal skin, yellow skin or yellow papules.

Binding activity of the paraprotein

Since paraproteins can bind lipoproteins (Kayden *et al.*, 1962; Beaumont *et al.*, 1965; Aubert *et al.*, 1967; Reisen *et al.*, 1971; Kodama *et al.*, 1972) the following study was done:

In an agar plate, two antigen holes (wells) were made and both filled with the patient's serum. After

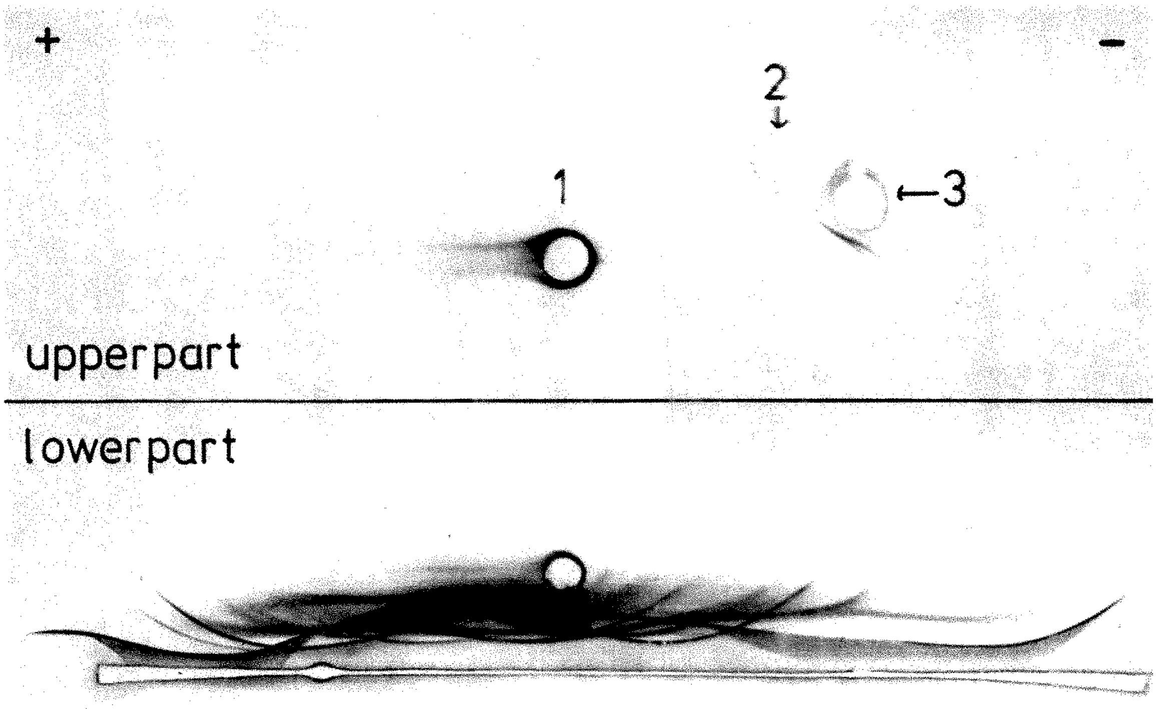

FIGURE 3. *Upper part*: After electrophoretic separation of patient's serum (hole 1), 2 holes were made above the γ-region. Hole 2 was filled with HDL, hole 3 with anti-HDL. Note the 2 precipitation lines showing complete identity. *Lower part*: Immunoelectrophoretic pattern of patient's serum developed with horse antihuman serum, showing an IgG paraprotein.

electrophoretic separation a trough was made below the lower well and filled with anti-human or anti-IgG serum (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam). The result showed clearly an IgG paraprotein (Fig. 3, lower part). On the upper part of the plate two holes were made above the region where the paraprotein is situated. One hole was filled with one of the serum lipoprotein fractions isolated from a healthy subject (e.g. HDL) and the other with the antiserum (anti-HDL (Behringwerke)). The results showed a precipitation line between a protein in the γ-region and the anti-HDL serum. This precipitation showed complete identity between the precipitation line formed by HDL and anti-HDL (Fig. 3, upper part).

No precipitation lines were formed between a protein in the γ-region and anti-VLDL (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam) or anti-LDL (Behringwerke).

Control experiments using serum of a normal subject and of a normolipidaemic patient with multiple myeloma showed no precipitation line between a protein in the γ-region and antisera against LDL, VLDL or HDL.

From these studies it can be concluded that a protein in the γ-region, probably the paraprotein, had bound HDL.

Detection of C_{1q} reactive material by C_{1q} precipitation

(Dr S.S.Asghar, Department of Dermatology, Binnengasthuis, Amsterdam.)

It is known that under proper conditions the C_{1q} component of serum complement will precipitate soluble immune complexes. Although other substances such as meningococcal group A polysaccharides, endotoxin lipopolysaccharides and DNA in large amounts can also precipitate with C_{1q}, the gel precipitation method described by Agnello, Winchester & Kunkel (1970) was found useful in detecting the presence of immune complexes.

Preparation of agarose gel plates under optimal conditions and isolation of C_{1q} were done as described by Agnello *et al.* (1970). Five wells were cut in the plate: the centre well was filled with C_{1q} (500 μ/ml), the others were filled respectively with normal serum, patient's serum, anti-C_{1q} (1 μ/ml) (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam) and DNA (200 μ/ml). A precipitation line formed between C_{1q} and patient's serum, anti-C_{1q} and DNA.

It can be concluded that complexes, precipitable by C_{1q}, are present in the serum of the patient. However, the exact nature of these complexes remains unknown; they can be either immune complexes or non-specifically aggregated γ-globulins.

DISCUSSION

Plane xanthomata seem to lack a specific histopathological picture. Bazex *et al.* (1965) claimed that no giant cells of either the Touton or the foreign-body type occur in these lesions, but Winkelmann & Welborn (1969), on the contrary, considered such giant cells to be a characteristic feature. Our patient did not show giant cells. However, we also found foam cells even in clinically normal skin, which to the best of our knowledge, has not been reported previously.

The xanthomatous skin had a relatively high triglyceride and a low cholesterol content. Kodama *et al.* (1972) patient with plane xanthomata, IgG monoclonal gammopathy, cryoglobulinaemia, and hyperlipoproteinaemia type IV, showed, on the contrary, a high cholesterol and a low triglyceride content. In neither case did the composition of the xanthoma reflect the cholesterol: triglyceride ratio in the blood. The difference between the observations of Kodama *et al.* and ours is probably due to the different ages of the xanthomata. Baes, van Gent & Pries (1968) demonstrated that the lipid composition of xanthomata is more closely related to the age of the xanthomata than to the lipoprotein content of the blood. Moreover, using 2-^{14}C acetate, Hu, Ellefson

& Winkelmann (1973) demonstrated *in vitro* that in plane xanthomata lipids can be synthesized *in situ*. The lipids we find in young xanthomata probably have permeated the vessel wall and are subsequently phagocytozed by macrophages. In the older lesions, due to the local synthesis of triglycerides, the lipid composition resembles that of tissue lipids.

The hyperlipidaemia in the case reported here is type IIA. The family investigation makes a genetically determined phenotype highly improbable and the hyperlipoproteinaemia was not secondary to a pancreatic, hepatic, or renal disease.

In multiple myeloma, serum lipid levels are usually low; they are very rarely elevated. This suggests that the paraproteins may interfere with lipoprotein metabolism. Paraproteins in multiple myeloma occasionally have antibody activity against several antigens (Zetterval *et al.*, 1966). Beaumont *et al.* (1965) were the first to demonstrate a binding activity of the paraprotein against LDL. They explained the hyperlipidaemia in their patient by a reduced catabolism of the paraprotein–lipoprotein complexes formed in the patient's serum. On the other hand, Riesen *et al.* (1972) found myeloma proteins with binding activity against LDL, associated with *hypo*lipidaemia. After treatment, disappearance of the paraprotein was accompanied in their case by a distinct increase of the serum lipids. The authors assumed a more rapid catabolism of the paraprotein–lipoprotein complexes.

In our case it seems very likely that the paraprotein had bound HDL but this does not explain the hyperlipoproteinaemia type IIA, which is on the contrary characterized by elevated LDL levels. Whether the binding of the paraprotein and HDL is specific, i.e. an antigen–antibody reaction, or non-specific needs further investigation.

It is not clear what immunological role the paraprotein plays in either the lipoprotein disturbance or the formation of plane xanthomata, but it seems possible that the paraprotein has auto-antibody activity against an unknown cutaneous lymphoreticular cell constituent, eliciting plane xanthoma as postulated by Feiwel (1968). However, the investigations on our patient did not reveal evidence of an organ-specific auto-immune disease.

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British Journal of Dermatology (1975) **93**, 417.

Dermo–epidermal adhesion and its effect on epidermal structure in the mouse

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Accepted for publication 28 January 1975

SUMMARY

Evidence is presented that dermo–epidermal adhesion is low in mouse ear. Consequently the pressure caused by mitotic activity in the basal epidermal layer is readily relieved by cell extrusion distally and the number of basal cells per unit skin area remains low. The rule then is that the number of distal cells per unit skin area must be equally low, and the epidermis is therefore thin.

Conversely, dermo–epidermal adhesion is high in mouse sole-of-foot epidermis, and the mitotic pressure leads through cell crowding to a columnar basal epithelium. Furthermore, in the footpad centre, where adhesion is evidently highest, the baseline folds. With basal cell crowding and baseline folding there are more basal cells, and therefore more distal cells, per unit skin area. The epidermis is thick.

Epidermal cells are regarded as equipotential. The different epidermal characteristics of the different body regions are determined by the combined actions of the local mitotic rate and the local strength of the dermal grip.

The complex relations between the epidermal mitotic rate and epidermal thickness were recently examined (Bullough, 1972, 1975). One conclusion was that an increasing mitotic rate is partly dependent on the production of increasing numbers of basal mitotic-cycle cells per unit skin area, which leads to basal cell crowding and thus to basal layer folding or doubling. Since the ratio, number of basal mitotic-cycle cells:number of distal post-mitotic cells, is a constant, this process also leads to the production of increasing numbers of distal keratinizing cells. Thus epidermal thickness increases.

The manner of increase in the number of basal mitotic-cycle cells is not yet adequately understood (Bullough, 1975), but it is at least partly dependent on the fact that each mitosis is usually so oriented that both the daughter cells remain basal (Bullough & Laurence, 1964). The pressure that a mitosis consequently causes within the basal layer is relieved when some closely adjacent cell, not in mitosis, is forced distally (Christophers, 1971), which suggests that a non-mitotic cell has a weaker dermal grip. A study has therefore been made of the part played by dermo–epidermal adhesion in the creation and relief of basal layer mitotic pressure, and of the effect this pressure has on epidermal structure.

MATERIALS AND METHODS

The mice

Different kinds of mice have different epidermal mitotic rates, and the ear epidermis of the following strains was examined: Strong CBA, Swiss A, Hewitt WHT/Ht and hairless hr/hr. The sole-of-foot epidermis of the CBA mice was also studied. Only adult males 3–4 months old were used and 4 h before killing each mouse was injected subcutaneously with 0·1 mg colcemid in 0·2 ml water. The injection, given at 11.00 h, arrested in the metaphase those mitoses that developed during the sleep period then beginning.

The epidermis

The skin, fixed in Bouin's fluid, was cut 7 μm thick and stained in Ehrlich's haematoxylin and eosin. The arrested mitoses were counted per cm length of sectioned epidermis and then, after the number of nuclei per mm of epidermal baseline had been determined, the figures were converted to mitoses per 1000 cells. The height:width ratio of the basal nuclei, used as a measure of basal layer pressure, was determined from photographs of random lengths of sectioned epidermis. The epidermal thickness, excluding the stratum corneum, was measured in μm. In all cases an average was established for each mouse and, in the tables, N represents the number of mice examined.

Induction of hyperplasia

Using Swiss A male mice, one ear was wetted daily on both sides by one drop of 1% retinoic acid in acetone (Christophers & Braun-Falco, 1968). Animals were killed after 4 days, after 6 days, or after 6 days followed by 3 days recovery.

RESULTS

Ear epidermis: normal and hypoplastic

The results obtained from the ear epidermis of the different strains of mice are shown in Table 1. The mitotic rates represent peak values reached during sleep periods, and although these rates differed by a factor of almost 2, there was no significant difference in the epidermal thickness. There was also no significant indication that the mitotic activity caused any build-up of pressure within the basal layer; in all groups the dermo–epidermal junction was flat, the numbers of nuclei per mm were similar, and the basal nuclei were almost spherical.

Bullough & Ebling (1952) have already described how a chronic reduction in mitotic activity causes no change in the thickness of mouse ear epidermis. In Table 2 their figures, converted to mitoses per 1000 cells per 4 h of the sleep period, show that even when the mitotic rate is reduced to less than a

TABLE 1. Data from the ear epidermis of various mouse strains

Epidermis (ear)	N	Mitoses/ 1000 cells/4 h	Nuclei/mm	Thickness (μm)	Nuclear height: width ratio
Swiss A	10	7·9 ± 0·91	128·1 ± 1·43	13·2 ± 0·88	0·97 ± 0·045
Strong CBA	15	9·1 ± 0·50	129·4 ± 1·83	12·9 ± 0·20	0·95 ± 0·023
WHT/Ht	14	12·7 ± 0·79	146·0 ± 1·71	13·1 ± 0·44	1·17 ± 0·043
Hairless	10	14·4 ± 0·99	147·6 ± 1·89	13·9 ± 0·52	1·09 ± 0·025

TABLE 2. Data from the ear epidermis of male CBA mice with various degrees of chronic mitotic depression induced by restricted diets*

Diets expressed as percentages of normal food intake	Mitoses/1000 cells/4 h (assuming 1300 cells/cm)	Epidermal thickness (μm)
55	3·46 ± 0·26	16·8 ± 0·2
65	3·54 ± 0·18	16·0 ± 0·7
75	5·85 ± 0·40	16·9 ± 0·6
85	12·77 ± 0·89	16·4 ± 0·6
95	15·00 ± 1·12	16·4 ± 0·2
105	15·92 ± 0·95	16·3 ± 0·3

* Calculated from Bullough & Ebling (1952): N = 5 throughout.

quarter of normal there is no shrinkage in the epidermal thickness. Evidently fewer basal cells are forced distally to age and keratinize more slowly; the reduced rate of cell production is equalled by the reduced rate of cell loss.

Such an epidermis is described as being in phase 1 (Bullough, 1972). However, above a certain critical mitotic rate the balance is lost and the epidermis begins to thicken; this is phase 2 in which epidermal thickness is directly proportional to the mitotic rate.

Ear epidermis: hyperplastic

To study the phase 2 situation, differing degrees of hyperplasia were created in the ears of Swiss A males using topically applied vitamin A acid. In such hyperplasia there is no diurnal rhythm, the high mitotic rate continuing unabated through both waking and sleeping periods (Bullough & Laurence, 1957). The ear epidermis was studied after a partial response (4 days treatment), a full response (6 days treatment), and a partial recovery (6 days treatment and 3 days recovery). The results, arranged in order of the mitotic rates, are in Table 3.

The full mitotic response reached almost × 17 the normal peak mitotic rate seen in sleeping Swiss A males; the epidermal thickness (excluding the stratum corneum) exceeded × 5 normal. However,

TABLE 3. Data from the ear epidermis of male Swiss A mice with various degrees of vitamin A acid-induced hyperplasia

State of epidermis	Mitoses/1000 cells/4 h	Nuclei/mm	Thickness (μm)	Nuclear height: width ratio
Normal	7·9 ± 0·91	128·1 ± 1·43	13·2 ± 0·88	0·97 ± 0·045
4 day hyperplasia	55·4 ± 6·28	127·5 ± 2·52	34·6 ± 1·22	1·18 ± 0·046
Partial recovery	101·1 ± 11·00	128·3 ± 2·07	57·7 ± 2·70	1·14 ± 0·086
6 day hyperplasia	132·7 ± 10·84	127·5 ± 2·17	69·4 ± 3·15	1·17 ± 0·032

N = 10 throughout.

TABLE 4. Proportions of basal to non-basal mitoses in various degrees of vitamin A acid-induced hyperplasia in the ear epidermis of male Swiss A mice

State of epidermis	Mitoses/1000 cells/4 h		
	Basal	Non-basal	Total
Normal	7·9 ± 0·91	0	7·9 ± 0·91
4 day hyperplasia	45·8 ± 5·08	9·6 ± 1·38	55·4 ± 6·28
Partial recovery	63·9 ± 6·75	37·2 ± 5·51	101·1 ± 11·00
6 day hyperplasia	78·1 ± 5·51	54·6 ± 5·54	132·7 ± 10·84

N = 10 throughout.

these dramatic changes evidently produced no increased lateral pressure in the basal layer; the dermo–epidermal junction remained relatively flat, the number of basal nuclei per mm section length was unchanged, and the nuclear height : width ratio remained almost normal (Fig. 1a, b).

An increased epidermal mitotic rate involves an increased number of mitotic-cycle cells per unit skin area (Bullough, 1972, 1975). In normal mouse ear epidermis all the mitoses are basal; in hyperplasia the extra mitotic-cycle cells are displaced distally to form a second, and in places even a third, layer (Fig. 1b). Table 4 shows that in moderate hyperplasia 17% of mitoses were non-basal, in partial recovery 37% were non-basal, and in full hyperplasia 41% were non-basal. However, the actual percentages of non-basal mitotic-cycle cells were probably higher than these figures suggest, since the farther cells are removed from the basal layer the less frequently they are likely to enter mitosis.

These results suggest that the basal cells of mouse ear epidermis are so readily detachable from the basement membrane that the lateral pressure generated by an increased mitotic rate is immediately relieved by cell displacement distally. Since so many of the displaced cells remain mitotic the epidermis then thickens in accordance with the ratio, number of mitotic cells : number of post-mitotic cells = a constant (Bullough, 1972). The increase in the number of post-mitotic cells occurs even though these cells keratinize and die more quickly than normal. Hyperplastic epidermis is also thicker because the damaged cells have greater than normal volume.

Sole-of-foot epidermis

This epidermis is thick, it has a tall columnar basal cell layer, and in the centre of each footpad it has a folded dermo–epidermal junction (Fig. 1c). The data obtained from the footpads of male CBA

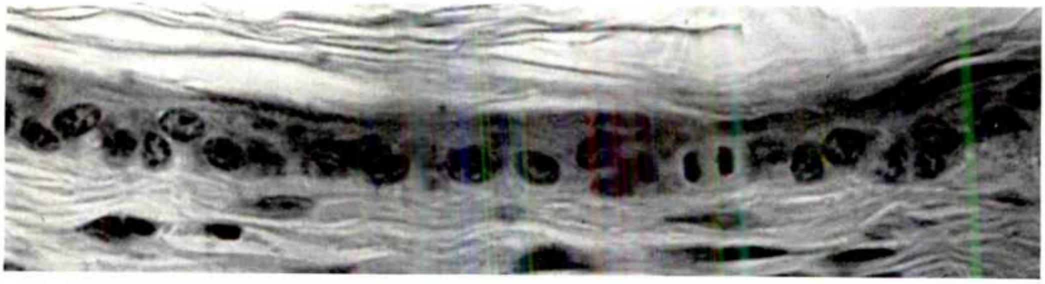

FIGURE 1a. Normal mouse ear epidermis showing the uncrowded basal cells and the horizontal orientation of mitosis (× 800).

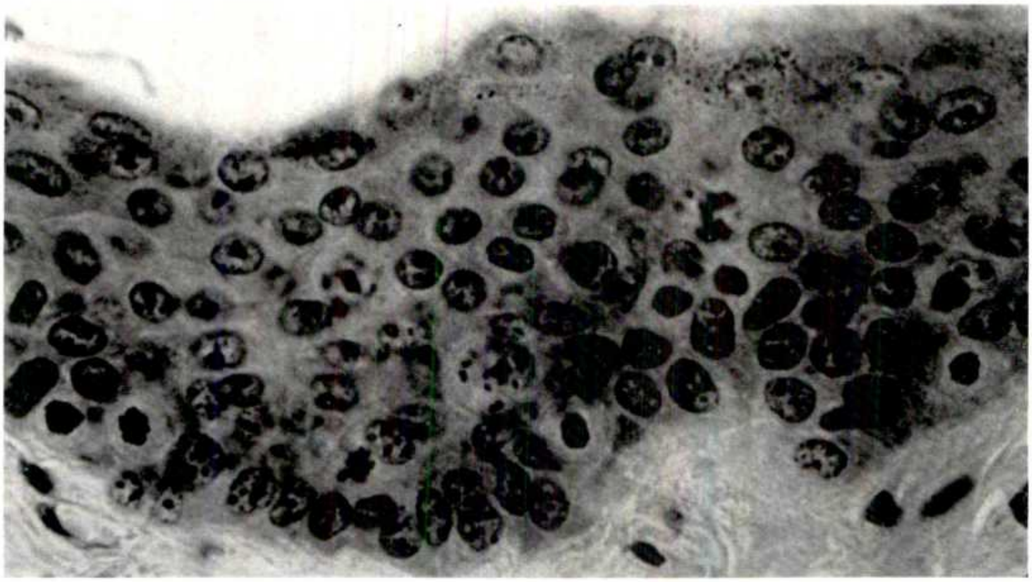

FIGURE 1b. Hyperplastic mouse ear epidermis showing the uncrowded basal cells, the non-basal mitoses, and the degree of thickening (× 800).

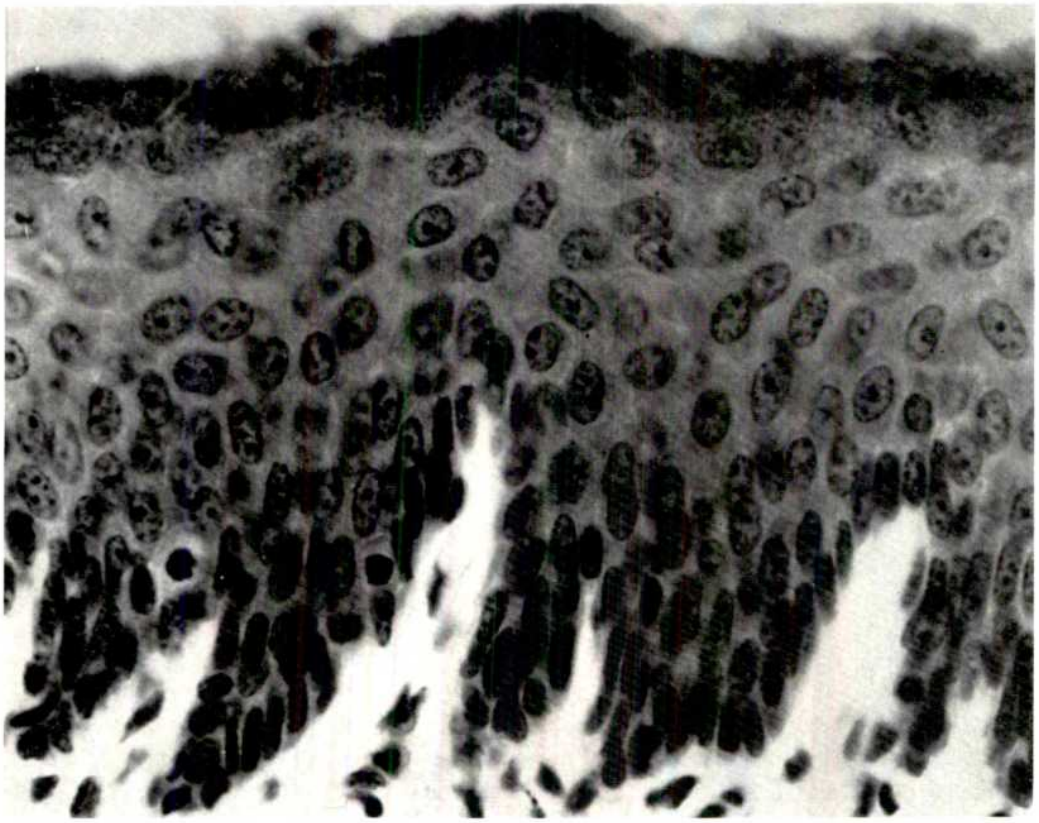

FIGURE 1c. Normal mouse sole-of-foot epidermis showing the crowded columnar basal and supra-basal cells and the folded baseline (× 800).

TABLE 5. Data from the ear and sole-of-foot epidermis of male CBA mice

Epidermis	N	Mitoses/1000 cells/4 h	Nuclei/mm	Thickness (μm)	Nuclear height : width ratio
Ear	15	9·1 ± 0·50	129·4 ± 1·83	12·9 ± 0·20	0·95 ± 0·023
Sole-of-foot:					
edge	10	14·6 ± 1·02	177·6 ± 2·46	40·6 ± 1·45	1·88 ± 0·057
centre	10	15·5 ± 1·54	324·2 ± 7·95	87·7 ± 2·57	3·85 ± 0·141

mice are shown in Table 5, and they are strikingly different from those obtained from the ear epidermis.

Although the mitotic rate is only slightly higher than that of hairless mouse ear epidermis (Table 1), the sole-of-foot epidermis is much thicker, the number of basal cells per mm is much greater, and the nuclear height:width ratio is a measure of the columnar shape of the cells. The indications of high lateral pressure within the basal layer are obvious: at the edge of the footpad, with a lower mitotic rate, the dermo–epidermal junction remains flat and the pressure is absorbed by the cell crowding; in the centre of the footpad, with a higher mitotic rate, the higher pressure is absorbed by greater cell crowding and by the folding of the dermo–epidermal junction.

In the footpad centre the mitotic rate is × 1·7 that of ear epidermis. The number of basal nuclei per mm is × 2·5 that of ear epidermis, but when the baseline folding is allowed for, the number of basal nuclei per unit area is × 5 that of ear epidermis. Theoretically the thickness of the footpad epidermis would be expected to show a similar increment whereas in fact it reaches almost × 7 that of the ear; part of this extra thickness is due to the columnar shape of the basal and suprabasal footpad cells.

When compared with hairless mouse ear epidermis (Table 1), it is clear that the lateral pressure in the basal layer of the sole-of-foot epidermis is not due simply to the mitotic rate. This becomes even clearer when the comparison is with the mouse ear hyperplasia in which the mitotic rate is far higher (Table 3). The only obvious explanation is that the pressure in sole-of-foot epidermis is due to the combined effects of the mitotic rate and the dermal grip on the basal cells. This grip seems to be strongest in the footpad centre.

DISCUSSION

The conclusion is that, in normal epidermis, the stronger the dermo–epidermal adhesion, the more crowded the basal cells, the more numerous the distal cells, and the thicker the epidermis. However, dermo–epidermal adhesion is evidently not constant; it varies throughout the mitotic cycle, being stronger during the mitotic process and weaker during part of the intermitotic interval. The mitotic process is initiated by a chalone-dependent trigger mechanism and it consists of the following phases: G1c (preparation for DNA duplication), S phase (DNA duplication), G2 (preparation for mitosis), mitosis itself, and G1a (for discussion see Bullough, 1975). In normal mouse epidermis it is observed that the S phase cells and the mitosis cells are always basal; it follows that no basal cells are displaced distally from the beginning of G1c to the end of mitosis. Frankfurt (1967) has also described how, in mouse forestomach keratinizing epithelium, the mitotic daughter cells do not begin to move distally until some 24 h after the end of mitosis, which indicates that G1a cells are also not displaced. Indeed, during the whole mitotic process, the only cell loss distally is from those few mitoses that are angled vertically (Bullough & Laurence, 1964).

Most, if not all, basal cell extrusion must be of cells in that part of the intermitotic interval called the dichophase (Bullough, 1963, 1975) or the G1b phase (Temin, 1971). In addition, in phase 1 type epidermis, the basal cells that are extruded may already have entered the early post-mitotic phase (Christophers, 1971).

The nature of dermo–epidermal adhesion is poorly understood and experiments designed to determine the force needed to separate a whole sheet of epidermal cells (Kiistala, 1972; Leun, Lowe & Beerens, 1974) provide little if any information regarding the manner of separation of a single dichophase cell. The epidermis appears to be held in place partly by the viscosity of a fluid dermo–epidermal interface (Leun *et al.*, 1974) and partly by an interdigitated cell–dermis boundary (Brody, 1960; Palade & Farquhar, 1965). In mouse ear epidermis the dichophase cell attachment becomes so weak that any pressure caused by a local mitosis is immediately relieved by the extrusion of the cell; in sole-of-foot epidermis the dichophase cell retains such a grip that it cannot be extruded until after the mitotic pressure has increased to such a level that the basal cells have become columnar and in the middle of the footpad the dermo–epidermal boundary has folded.

This conclusion provides an explanation for the results of Billingham & Silvers (1968), who found that, when mouse ear epidermis and sole-of-foot epidermis are transposed, each changes its structure to resemble that typical of the graft site (contrast Fig. 1a with 1c). They drew the important conclusion that the different types of epidermis in the different body regions 'are equipotential and that the maintenance of their particular epidermal specificities turns upon the persistent influence of some kind of inductive stimulus from the underlying dermis'.

Dermal control of epidermal specificity is now seen to depend not so much on an 'inductive stimulus', which has an embryological connotation, but on two dermal factors. The first, recently discussed by Bullough (1975), is a mitosis-promoting 'mesenchymal factor' which, diffusing from the dermis into the basal epidermal layer, determines the mitotic rate; the second is the grip of the dermis on the epidermis. Ear epidermis, when transplanted on to sole-of-foot dermis, evidently encounters a higher mesenchymal factor concentration and is held in a stronger dermal grip. The result is that the mitotic rate increases, the basal cells become more crowded, and the whole epidermis thickens. Sole-of-foot epidermis, transplanted on to ear dermis, changes in the opposite direction.

Thus epidermal structure is dermis-dependent. Functionally, sole-of-foot epidermis must be more tightly attached than other body epidermis in order to resist shearing forces; the tighter grip then results in a thicker epidermis which is better able to resist abrasion and damage. Clearly, before a full understanding of the differing structures of the various epidermal types can be obtained, much further information is needed on the differing activities of the various dermal types.

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The differential binding of antibody from the sera of patients with pemphigus and pemphigoid to isolated guinea-pig epidermal cells

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Accepted for publication 28 January 1975

SUMMARY

A method for preparing isolated epidermal cells using trypsin is described. The isolated cells retain the ability to bind antibody from patients with pemphigus or with pemphigoid in a disease-specific manner.

The finding by Beutner & Jordon (1964) of antibodies in the sera of patients with pemphigus which reacted with the intercellular substance of xenogeneic epidermis, focused attention on an immunological involvement in this disease. Further work demonstrated that the antibody titre correlated with disease activity and proved a useful index for predicting exacerbations (Chorzelski, Von Weiss & Lever, 1966; Beutner, Jordon & Chorzelski, 1968; Peck *et al.*, 1968). Patients suffering from pemphigoid were also found to have antibodies which reacted with the skin. The specificity of the antibodies, however, was directed against determinants in the region of the basement membrane rather than the intercellular substance (Beutner *et al.*, 1965; Jordon *et al.*, 1967) and the antibody titre appeared to be less related to disease activity (Jordon *et al.*, 1967; Peck *et al.*, 1968).

Although the antibodies in both pemphigus and pemphigoid have been shown to be disease specific, little information is available on the nature of the antigen. Recently, a protein of molecular weight 68,000 has been extracted from human and rabbit oesophagus and has been shown to be capable of absorbing pemphigus antibody from the sera of patients with the disease (Shu & Beutner, 1973). This suggests that the protein may have antigenic properties.

A major factor limiting the investigation into the antigenic specificity of these antibodies is the lack of a suitable model system which would allow detailed analysis of the antigen–antibody binding. The work reported in this paper characterizes the binding of serum antibody from patients with pemphigus or pemphigoid to isolated guinea-pig epidermal cells. It was found that by using an indirect immunofluorescence technique, distinct staining patterns could be observed. The patterns were reproducible and specific for the disease. It is hoped that the investigation of antibody binding to isolated cells may prove a suitable model for studying determinant specificity.

MATERIALS AND METHODS

Animals. Dunkin Hartley outbred guinea-pigs were used throughout.

Test sera. Sera were used from patients with clinically, histologically, and immunofluorescently proven pemphigus and pemphigoid. A pool of control serum was obtained from normal healthy laboratory workers.

Anti-globulin reagents. Sheep anti-human immunoglobulin FITC labelled (Wellcome Batch No. K7247) F/P ratio 3·9 (protein 5·8 mgs/ml) was used at a dilution of 1/30.

Sheep anti-human immunoglobulin (Wellcome Batch No. 7908) was used as a blocking reagent.

Microscope. The fluorescence was assessed using a Reichert Zetopan Fluorescence Microscope fitted with an HBO 200 mercury vapour lamp and using a BG 12/6 mm exciter filter and GG9/1 mm and OG 1/1·5 mm absorption filters.

Trypsin. Crystalline trypsin from beef pancreas (activity 7500–8000 BAEE units/mg).

Medium. Eagles', minimal essential medium (Wellcome) +30% fetal calf serum (Biocult).

Methods. Existing techniques for preparing epidermal cells from guinea-pigs (Cruickshank, Cooper & Hooper, 1960; Aoki, Parker & Turk, 1970) were modified. Guinea-pig ears were removed and the hairs shaved in 0·1% Hycolin solution. The skin was then separated from the underlying cartilage by blunt dissection with scissors and the remaining cartilage was removed with a scalpel. The skin was cut into strips 2–4 mm wide, washed rapidly in 70% alcohol and immersed in 0·3% trypsin solution in phosphate buffered saline (PBS) for 45 min. The action of trypsin was then stopped by removing the strips and reincubating them for a further 15 min in culture medium. This procedure allowed the thin keratin layer to be removed with fine forceps, releasing dissociated epidermal cells into the medium. The remaining attached cells were gently freed by scraping the epidermal surface with forceps.

The epidermal cell suspension was filtered through gauze and the cells centrifuged at 100 *g* for 5 min. The cells were washed with PBS and recentrifuged as above. Two drops of the suspension were then placed on an alcohol cleaned coverslip and air dried at room temperature.

Tissue sections. A composite block of guinea-pig lip and oesophagus was prepared and snap frozen in liquid nitrogen. 4μm sections were cut and placed on alcohol cleaned coverslips and air dried for 60 min.

Fluorescence staining. Standard techniques were used (Coons & Kaplan, 1950; Weller & Coons, 1954; Beutner, Chorzelski & Jordon, 1970) and cell preparations were treated with either test or control sera for 30 min at room temperature. The preparations were then washed and stained with FITC labelled antiglobulin. In blocking experiments an unlabelled antiglobulin was applied before the labelled material. Each application was allowed to proceed for 30 min followed by a 30 min wash before proceeding to the next step. A similar procedure was used to test epidermal cells in suspension. The cells were washed with PBS between each step by centrifuging at 100 *g* for 5 min. A drop of the cell suspension was then placed on a glass slide, covered with a cover slip and sealed with wax. All results were assessed by an observer with no prior knowledge of the nature of the test sera applied.

RESULTS

The number of cells obtained from one pair of ears was in the region of 10^7. It proved possible by careful manipulation to maintain the cells in single suspension without undue clumping.

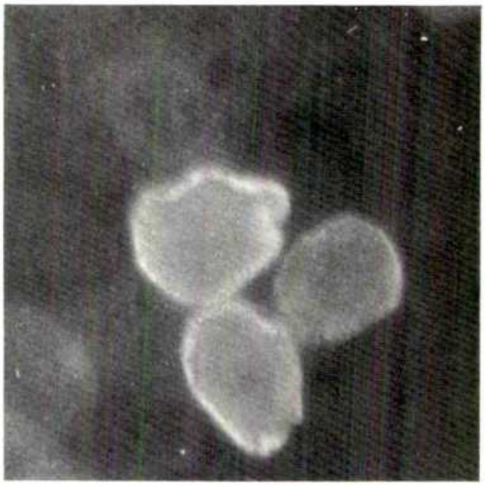

FIGURE 1(a).

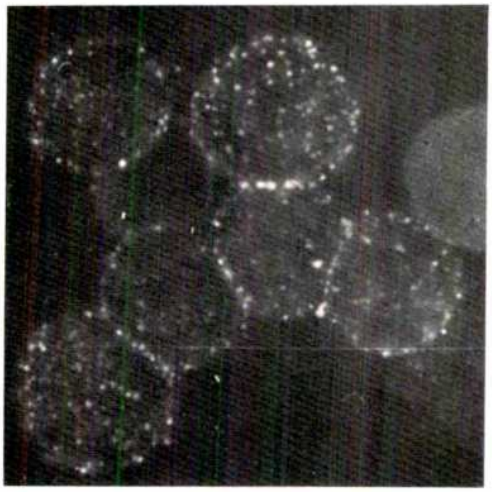

FIGURE 1(b).

FIGURE 1. Immunofluorescent staining of isolated epidermal cells treated with serum from pemphigus patients. (a) Cells air dried. (b) Cells stained in suspension.

Fig. 1(a) shows the appearance of the air-dried cells treated with serum from a patient with pemphigus and stained with FITC polyvalent antiglobulin. It can be seen that a ring of fluorescence extends continuously around the periphery of the cells. The number of cells within a given suspension that stained in this way was about 30%. The remaining cells did not show the ring fluorescence but the cytoplasm was weakly stained and appeared as a faint background fluorescence similar to the control cells treated with normal human serum. Fig. 1(b) shows the appearance of the cells treated in suspension with pemphigus serum. A rather different appearance is seen showing a granular pattern at the periphery.

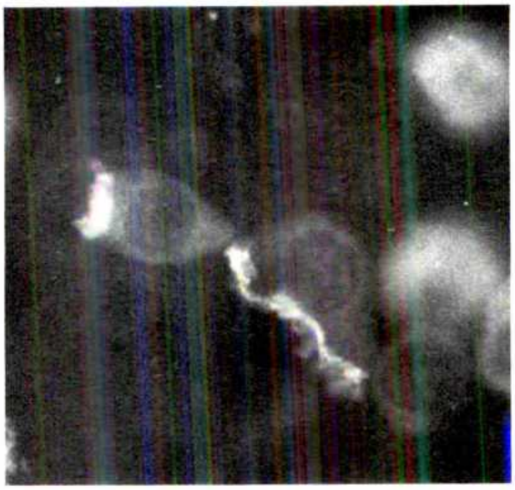

FIGURE 2(a).

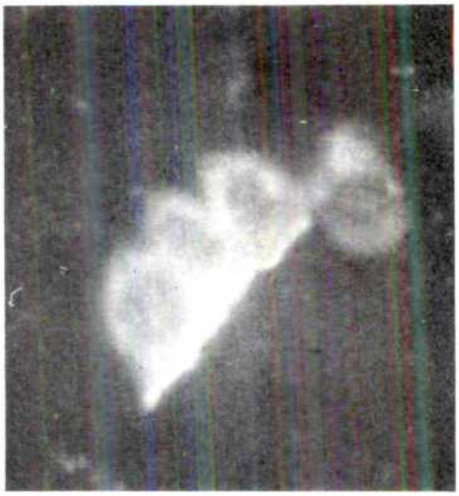

FIGURE 2(b).

FIGURE 2. Immunofluorescent staining of isolated epidermal cells treated with pemphigoid serum. (a) Isolated cells. (b) Staining pattern on three attached cells.

Treating either air-dried cells or cells in suspension with serum from patients with pemphigoid and staining in the same way then showed fluorescence confined to one pole (Fig. 2a). If two or more cells were joined together the staining appeared continuous along the cells but was still confined to one margin (Fig. 2b). Sera taken from six pemphigus and six pemphigoid patients and tested on the isolated cell suspensions confirmed that the staining patterns were reproducible.

The antibody titre, as determined by using separated epidermal cells, compared well with that obtained by using tissue sections. The results are shown in Table 1. It can be seen that the titre obtained with pemphigoid and with pemphigus sera was similar on both substrates.

The specificity of the staining was checked by inhibition experiments using unconjugated antihuman globulin (Table 2). Complete blocking of the immunofluorescent pattern was achieved on tissue sections and on cells at an antibody titre of 1/80. Partial inhibition, i.e. a considerable reduction in the intensity of the staining, was noted at a serum titre of 1/10.

TABLE 1. Comparison between guinea-pig lip/oesophagus preparation and isolated cells for measuring the titre of pemphigus and pemphigoid antibody

		Titre					
Serum	Substrate	1/10	1/80	1/160	1/320	1/640	1/1280
Pemphigus	Tissue	+++	++	+	–	–	–
	Cells	+++	++	–	–	–	–
Pemphigoid	Tissue	++	++	++	+	+	–
	Cells	+++	++	++	+	+	–
Normal	Tissue	–	–	–	–	–	–
	Cells	–	–	–	–	–	–

Scoring of fluorescence: + Faint fluorescence; ++ Clear discrete fluorescence; +++ Very bright discrete fluorescence; – No fluorescence.

TABLE 2. Blocking effect of antihuman globulin on the binding of FITC labelled antiglobulin to cells and tissues

	Pemphigus				Pemphigoid			
	Tissue		Cells		Tissue		Cells	
Titre	Control	Blocked	Control	Blocked	Control	Blocked	Control	Blocked
1/10	+++	+	+++	++	++	–	+++	–
1/80	++	–	++	–	++	–	++	–
1/160	+	–	–	–	++	–	++	–
1/320	–	–	–	–	+	–	+	–

These results confirm that the antigenic determinants which react with the antibody found in the serum of patients with pemphigus and pemphigoid are present on isolated epidermal cells. Furthermore there is no loss of antigenic activity, as determined by antibody titre, due to the procedures used to separate the cells.

DISCUSSION

The fact that humoral antibodies found in patients with pemphigus and pemphigoid react with xenogeneic epidermis points to a considerable homology between epidermal structure in different species. This justifies the use of guinea-pig epidermal cells as a model for investigating the antigenic specificity of these two antibodies.

Our results show that trypsinized epidermal cells taken from guinea-pig ears can be used to demonstrate serum antibodies in patients with pemphigus and pemphigoid. Furthermore, the staining patterns of the sera are quite distinct and can be used to differentiate between the two diseases.

It is perhaps surprising that trypsin treatment does not affect antigenic structure, particularly as the antigen reacting with the pemphigus antibody is thought to be a protein. One possible explanation of this would be that the concentration of the trypsin used during preparation of the cells was insufficient to digest the antigenic determinants. Equally acceptable is the possibility that the determinants might not be trypsin sensitive. This could well be the case if the determinants are the non-protein moiety of epidermal cell wall glycoprotein. Since the antibodies react with separated cells it is likely that the antigenic determinants are intimately associated with the cell membrane and not related to the integrity of the intercellular substances or basement membrane. In our studies it was not possible to exclude attachment of basement membrane to epidermal cells as an explanation of the polar staining seen when the cells were treated with serum from pemphigoid patients. Fukuyama, Black & Epstein (1974), have shown, however, that the process of separating epidermal cells from the dermis using trypsin also separates them from the basement membrane. Further experiments are in progress to obtain more definite information on the nature of the antigen.

ACKNOWLEDGMENTS

Dr W.D.Brigden is in receipt of an MRC Clinical Research Fellowship which is gratefully acknowledged. The authors would like to thank Miss Patricia Maher, B.A. for her careful technical assistance.

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British Journal of Dermatology (1975) **93**, 431.

Immunohistological studies, with anti-connective tissue and anti-immunoglobulin antisera, of the skin in lupus erythematosus and scleroderma

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Accepted for publication 2 January 1975

SUMMARY

Skin lesions from six patients with systemic lupus erythematosus, five patients with discoid lupus erythematosus, twelve patients with systemic sclerosis, five patients with localized morphoea and twenty controls were examined by immunohistological techniques using fluorescein-labelled anti-human IgG, anti-human C3 and anti-human renal glomerulus antisera.

The major immunohistological changes in systemic sclerosis and in localized morphoea consisted of foci of intercollagenous staining for connective tissue antigens in the reticular layer of the dermis. It is suggested that these findings indicate collagen neogenesis.

In lupus erythematosus the major changes occur in the dermo-epidermal junction and consist of deposits of IgG and C3 and thickening and disruption of the membrane as demonstrated by the use of heterologous sera containing antibasement membrane antibodies.

Immunohistological techniques are useful in the diagnostic differentiation between scleroderma and lupus erythematosus.

The deposits of immunoglobulin and complement found at the dermo-epidermal junction and in papillary and subpapillary blood vessels in the skin lesions of systemic lupus erythematosus (SLE) and discoid lupus erythematosus (DLE) have been taken to be toxic immune complexes which play a part in the pathogenesis of the lesions (Cream & Turk, 1971).

The study reported here was undertaken to determine whether the deposits could be related to morphological abnormalities of connective tissues or blood vessels as revealed by the use of a heterologous antiserum containing antibodies to basement membrane and reticulin.

It was felt worthwhile to use the same approach in a study of the lesions of the two forms of scleroderma—systemic sclerosis (SS) and localized morphoea (LM)—since immune mechanisms, abnormalities of connective tissue metabolism and vascular disturbances have all been postulated as aetiological or pathogenetic processes in these diseases (Shulman, 1969).

MATERIALS AND METHODS

Skin lesions were obtained at autopsy or biopsy from six patients with SLE, five with DLE, twelve with SS and five with LM. Twenty specimens of skin from normal subjects were also examined.

Sections were cut in a cryostat from quick frozen unfixed blocks and processed by standard immunohistological techniques. Tests for immunoglobulins and complement were carried out on sections which had been washed for 45–60 min in phosphate buffered saline (0·05M PO_4 pH 8·0) and fixed for 1 h in 95% ethyl alcohol. Untreated sections were used for the detection of connective tissue antigens. The direct staining procedure was used for the detection of immunoglobulins and connective tissue antigens and the indirect for the detection of complement. Tests for specificity of staining were carried out according to Nairn (1969).

The reagent used for the detection of connective tissue antigens was a fluorescein-labelled globulin fraction of pooled rabbit anti-human renal glomerulus antisera. Methods used in the preparation of the antisera and conjugate have been previously described (Scott, 1957, 1959). Monovalent fluorescein-labelled anti-IgG, IgM and IgA antisera and unlabelled anti-C3 antisera were used for the detection of the corresponding antigens. These were obtained commercially from various sources (Scott & Rowell, 1974).

RESULTS

1. *Normal skin*

A. *Anti-basement membrane and reticulin conjugate.* The pattern of staining produced at the dermo-epidermal junction in normal skin by the fluorescein-labelled anti-renal glomerulus (FARG) conjugate is illustrated in Figs 1 and 2. The dermo–epidermal boundary membrane was seen as a fine single line, or as a tubular or finely serrated band. The pattern of staining varied with the plane of section.

Spiral capillaries in the dermal papillae showed thick fluorescent walls fenestrated by unstained spaces.

The conjugate also reacted with the basement membranes of sweat glands and their ducts, with the media of arteries and arterioles and with the neurolemma and perineurium of nerves.

The media of arteries and arterioles stained either as a reticulate network or as a series of concentric lamellae. These structures and the neurolemma and perineurium of nerves presented the same pattern of staining as the corresponding structures elsewhere in the body (Cruickshank & Hill, 1953).

The FARG conjugate produced no staining of collagen, intercollagenous spaces or elastic tissue.

B. *Anti-immunoglobulin and anti-C3 antisera.* Extravascular IgG was frequently found on collagen fibrils in the papillary layer of the dermis in washed and fixed sections of normal skin. A fine bright line of fluorescence for IgG was seen at the dermo–epidermal junction in 4 of the 20 biopsies of skin examined from normal subjects. This differed from the granular band-like pattern seen in lupus erythematosus (see below) and from the tubular pattern seen in pemphigoid. The walls of capillaries, arterioles and arteries showed no staining.

C3, IgA and IgM were not detected in sections of normal skin.

2. *Skin lesions*

The distribution and nature of the immunohistological changes found in skin lesions are summarized in Table 1 and set out in detail in Tables 2 and 3. Table 1 shows that three classes of change were encountered; (a) changes found only in SS and LM, (b) changes found mainly in SLE and DLE, and (c) changes common to both groups of diseases.

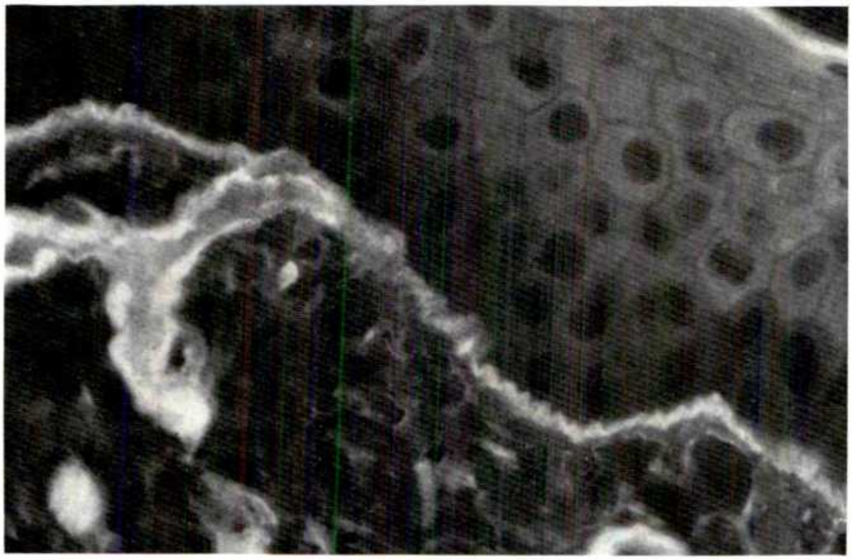

FIGURE 1. Normal human skin stained with the FARG conjugate. Staining appears in the dermo–epidermal boundary membrane and in sub-epidermal capillaries. The membrane presents a serrated appearance (× 700).

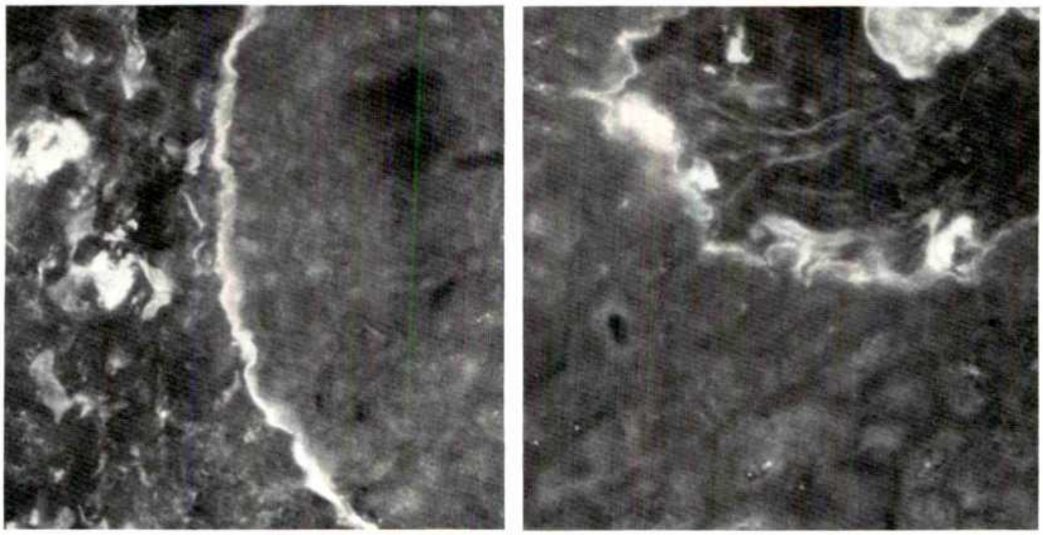

FIGURE 2. Normal human skin treated as in Fig. 1. Left—the dermo–epidermal boundary membrane presents in part as a single line and in part as a narrow tubular structure. Right—the membrane is seen as a single fine line (× 400).

TABLE 1. Frequency of immunohistological changes in the skin lesions of systemic and discoid lupus erythematosus, systemic sclerosis and localized morphoea

		SLE	DLE	Systemic sclerosis	Localized morphoea
		10	5	16	5
DEJ	M	7	5	0	0
	G	10	5	2	0
	C	7	2/3	0	0
	NEG	0	0	14	5
Capillaries (papillary and sub-papillary)	M	5	2	9	4
	G	7	2	3	2
	C	5	2/3	0	0
	NEG	3	1/3	6	1
Arterioles and arteries (tunica media)	M	2	2	14	0
	G	2	0	5	0
	C	1	0/3	0	0
	NEG	8	1/3	2	5
Collagen (papillary layer)	M	6	4	9	1
	G	2	3	4	0
	C	0	0/3	0	0
	NEG	4	1/3	6	4
Intercollagenous staining (reticular layer)	M	0	0	11	3
	G	0	0	1	0
	C	0	0/3	0	0
	NEG	10	3/3	5	2

DEJ, Dermo–epidermal junction; M, Abnormal morphological appearances with FARG conjugate; G, Staining for IgG; C, Staining for C3.

(a) *Changes found only in SS and LM.* These were found in the reticular layer of the dermis in sections stained with the FARG conjugate. They consisted of foci of fibrillar intercollagenous staining accompanied by widening of the intercollagenous spaces and thickening, splitting or fragmentation of elastic tissue (Fig. 3). The foci were never numerous, sections showing 1 or, at the most, 2 foci. They were present in 12 of the 16 SS lesions and in 3 of the 5 LM lesions examined. Intercollagenous staining for IgG was seen in 1 SS lesion but staining for C3 between collagen bundles was not encountered.

IgG was demonstrated at the dermo–epidermal junction in only 2 sections but this was of the thin bright line type seen in normal skin. Complement (C3) was not found at any site. Sections from 4 SS lesions were reacted with anti-IgM and anti-IgA conjugates and showed no staining.

(b) *Changes found mainly in SLE and DLE.* Morphological alterations in the boundary membrane were revealed by the use of FARG conjugates (Fig. 4). These were seen in 7 out of 10 SLE lesions, in all DLE lesions but not in sections of SS. In SLE and DLE they consisted of thickening or disruption of the membrane. In areas of thickening the membrane was seen as a broad, coarsely serrated or homogeneous band and diffusion of staining into the underlying tissues was sometimes present. In areas of disruption, boundary membrane staining was interrupted by unstained gaps or replaced by small fluorescent droplets.

Table 1 shows that immunoglobulins were regularly found at the dermo–epidermal junction in SLE and DLE but complement was detected at this site in only 7 SLE and 2 DLE lesions. Staining

TABLE 2. Distribution of immunohistological changes in the skin in systemic and discoid lupus erythematosus

Disease	Patient	DEJ			Capillaries (papillary & sub-papillary)			Arterioles & arteries (tunica media)			Collagen (papillary layer)			Inter-collagenous staining (reticular layer)			Type of lesion
		M	G	C	M	G	C	M	G	C	M	G	C	M	G	C	
SLE	1a	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	Reticulate
	b	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	Erythematous
	c	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	Atrophic
	2a	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	Purpuric
	b	+	+	+	–	+	–	–	–	–	+	–	–	–	–	–	Erythematous
	c	–	+	–	+	+	+	–	–	–	+	–	–	–	–	–	Reticulate
	3	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	Erythematous
	4	+	+	+	+	+	+	–	–	–	+	+	–	–	–	–	Erythematous
	5	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	Adjacent to ulcer
	6	+	+	+	–	+	–	–	–	–	+	+	–	–	–	–	Erythematous
DLE	7	+	+	ND	–	–	ND	–	–	ND	+	+	ND	–	–	ND	Erythema & oedema
	8	+	+	ND	–	–	ND	–	–	ND	+	–	ND	–	–	ND	Scaling erythema
	9	+	+	–	+	+	+	+	–	–	+	+	–	–	–	–	Scaling erythema
	10	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	Scaling erythema
	11	+	+	+	–	–	–	–	–	–	+	+	–	–	–	–	Scaling erythema

DEJ, Dermo-epidermal junction; M, Abnormal morphological appearances with FARG conjugate; G, Staining for IgG; C, Staining for C3.

TABLE 3. Distribution of immunohistological changes in the skin in systemic sclerosis and localized morphoea

Disease	Patient	DEJ			Capillaries (papillary & sub-papillary)			Arterioles & arteries (tunica media)			Collagen (papillary layer)			Inter-collagenous staining (reticulate layer)		
		M	G	C	M	G	C	M	G	C	M	G	C	M	G	C
Systemic sclerosis	12	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−
	13	−	−	−	+	−	−	+	−	−	−	−	−	+	−	−
	14a	−	−	−	+	−	−	+	−	−	+	−	−	+	−	−
	b	−	−	−	+	−	−	+	−	−	+	−	−	+	−	−
	15	−	−	−	+	−	−	+	−		+	−	−	+	−	−
	16	−	−	−	−	−	−	+	−	−	+	−	−	+	−	−
	17	−	−	−	+	−	−	+	+	−	−	−	−	−	−	−
	18a	−	−	−	−	+	−	+	+	−	+	+	−	+	−	−
	b	−	+	−	+	+	−	+	+	−	+	+	−	−	−	−
	19	−	−	−	+	−	−	+	−	−	−	−	−	+	−	−
	20	−	−	−	−	−	−	+	+	−	−	−	−	+	−	−
	21a	−	−	−	−	−	−	+	−	−	+	−	−	−		
	b	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
	22	−	+	−	+	+	−	+	+	−	+	+	−	+	+	−
	23a	−	−	−	+	−	−	+	−	−	−	+	−	+	−	−
	b	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Localized morphoea	24	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	25	−	−	−	+	+	−	−	−	−	−	−	−	+	−	−
	26	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−
	27	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
	28	−	−	−	+	+	−	−	−	−	−	−	−	+	−	−

DEJ, Dermo-epidermal junction; M, Abnormal morphological appearances with FARG conjugate; G, Staining for IgG; C, Staining for C_3.

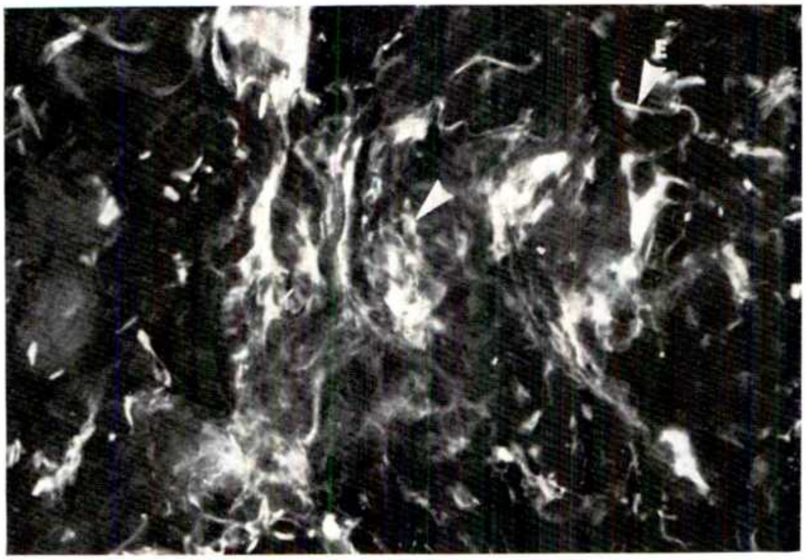

FIGURE 3. Systemic sclerosis skin (Case 20) with the FARG conjugate. Reticular layer of the dermis. There is a focus of intercollagenous staining (unlettered arrows). Elastic tissue (E) is seen as white fibrils or rods (× 64).

for IgG, and for C3 when present, was distributed either as a continuous homogeneous or granular band (Fig. 5) or as large, closely packed, aggregates.

(c) *Changes common to SLE, DLE, SS and LM*. Changes common to both groups of diseases involved: (a) connective tissues of the papillary layer of the dermis, (b) papillary and sub-papillary capillaries and (c) arterioles and arteries.

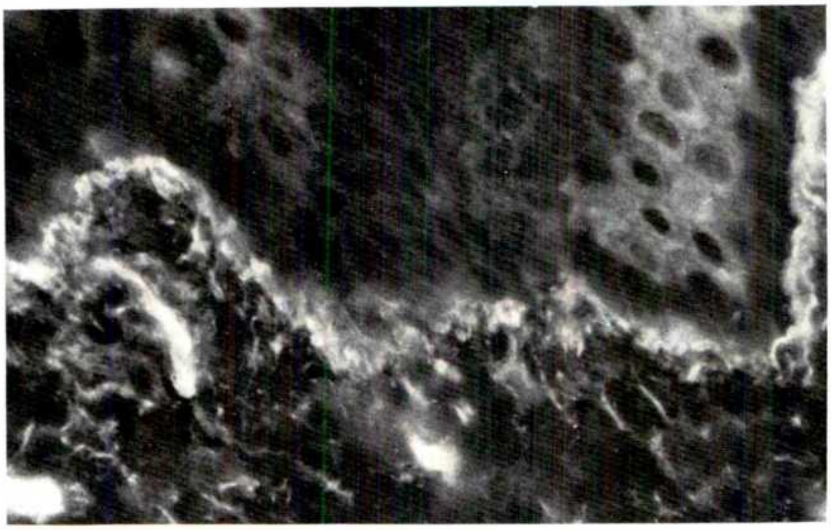

FIGURE 4. Broadening and disruption of the dermo–epidermal boundary membrane in an SLE lesion (Case 4). FARG stain (× 540).

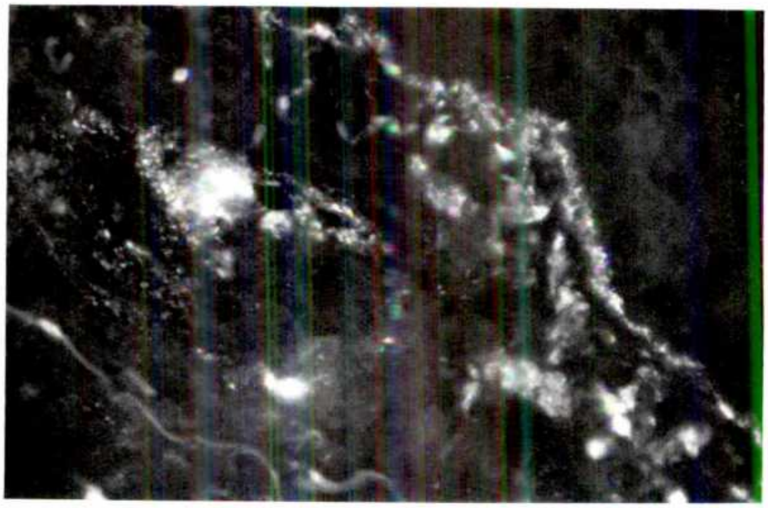

FIGURE 5. SLE skin lesion (Case 4) stained for C3. Granular staining is present in and around the wall of a capillary and at the dermo-epidermal junction (× 250).

(a) Extravascular deposits of IgG on collagen fibrils in the papillary layer of the dermis and fibrillar staining of these tissues with the FARG conjugate occurred with similar frequency in both groups of diseases (Table 1).

(b) Morphological abnormalities of capillaries, thickening of their walls and smudging or obliteration of their fenestrations (Fig. 6), were seen in FARG stained sections from 13 of 21 SS and LM lesions and from 7 of 15 SLE and DLE lesions. In contrast IgG was found in walls of capillaries in 7 of 10 SLE lesions and in 7 of 26 SS, LM and DLE lesions. C3 was demonstrated in the capillaries in 5 of the SLE and in 2 of the DLE lesions, but was not seen in SS or LM.

In SLE, capillaries showed the patterns of staining for IgG described by Baart de la Faille Kuyper Cormane (1968)—homogeneous; intramural granular; perivascular granular. A single capillary

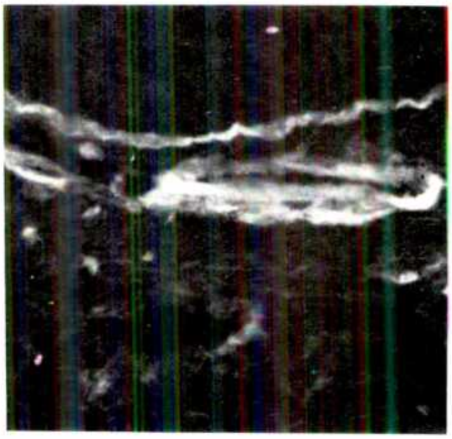

FIGURE 6. Smudging of the wall of a sub-epidermal capillary. Systemic sclerosis skin (Case 18). FARG stain (× 400).

showing all three patterns was occasionally seen. Only the homogeneous pattern was found in DLE, SS or LM.

(c) The morphological changes involving the intima and media of the arteries and arterioles were seen in FARG stained sections in 14 SS lesions and in 2 SLE and in 2 DLE lesions. The changes were similar to those found in renal vessels in SS (Scott & Rowell, 1974). They were accompanied by intramural deposits of IgG in 5 SS and 2 SLE lesions and by intramural deposits of C3 in one SLE lesion.

DISCUSSION

It has recently been suggested that the similarities between the clinical and laboratory features in patients with SLE and in those with SS raise the possibility that SS is, like SLE, an immune complex disease, perhaps one in which the blood vessels are involved primarily (Germuth & Rodriguez, 1973). The literature provides little support for this concept (e.g. Shulman, 1969) and our studies have yielded no evidence for a continuing role for immune complexes in the pathogenesis of the skin lesions of SS and LM.

The major immunohistological changes seen in SS and LM occurred in the reticular layer of the dermis. They consisted of foci of intercollagenous staining for connective tissue antigens and splitting, broadening or fragmentation of elastic tissue. Such foci were not seen in normal or in LE skin. Their presence in SS and LM may indicate collagen neogenesis. Although our FARG conjugate did not react with mature collagen, conjugates of the same specificity have been shown to react with fibroblasts and proliferating fibrous tissue (Scott, 1959) and it is possible that collagen neogenesis is associated with the appearance of antigens not detectable in the mature fibre. Soluble and insoluble collagens have been found to differ antigenically from each other (Paz *et al.*, 1963; Steffen, 1965). Immunohistological studies of the dermis in embryonic and adult rats have indicated that rapidly growing tissue in which collagen formation is taking place contains antigens not detectable in mature collagen (Scott & Rowell, 1967).

Features pointing to increased collagen synthesis have been noted in sclerodermatous skin by a number of workers. Fine filaments with repeated dense bands lying near fibroblasts and thin collagen bundles having embryonic characteristics have been described in electron microscopic studies of sclerodermatous skin (Kobayasi & Absoe-Hansen, 1972; Fleischmajer, Damiano & Nedwich, 1972; Braun-Falco & Rupec, 1964). Biochemical studies reviewed by Uitto (1971) have shown increased protocollagen proline hydroxylase activity. Leroy (1972) described increased activity of sclerodermatous fibroblasts in tissue culture. Although the dermis in SS and LM looks inert histologically it has been suggested that there may be differences in disease activity in different areas of skin (Uitto, 1971). This would accord with our finding of focal changes.

The view that the dermal changes in SS are secondary to vascular changes has been repeatedly advanced (Ingram, 1958; Norton & Nardo, 1970). In our studies morphological changes were found in arteries and arterioles in 14 of 16 SS lesions but they were not found in LM. These findings perhaps suggest that if SS and LM are related their common ground lies in connective tissues. Changes in papillary and sub-papillary capillaries, on the other hand, were found with equal frequency in SS and LM. It is, however, not clear how these changes relate to the main sites of collagen alteration which appear to involve deep dermal (Kobayasi & Absoe-Hansen, 1972) or subcutaneous tissues (Fleischmajer *et al.*, 1972).

Our studies of LE lesions throw no light on the part played by immune complexes in the pathogenesis of these lesions. Although we found a good correlation to exist between the presence of IgG and C3 at the dermo-epidermal junction and the presence of epidermal basement membrane abnormalities demonstrated by the use of heterologous sera containing anti-basement membrane antibodies in the erythematous, purpuric and atrophic lesions studied, it is possible that basement membrane abnormalities preceded the deposition of IgG and C3. In two reticulate lesions and in intact skin adjacent to an ulcerated lesion no basement membrane abnormalities were detected, although junctional deposits of IgG were found in the reticulate lesions and deposits of IgG and C3 in the third biopsy (Table 3). This may suggest that the pathogenic mechanisms involved in reticulate lesions differ from those involved in erythematous lesions.

Our observations suggest that immunohistological techniques can contribute to the diagnostic differentiation between SS and LM on the one hand and SLE and DLE on the other. Changes seen in SS and LM occurred in the reticular layer of the dermis and consisted of focal intercollagenous staining for connective tissue antigens. In SLE and DLE the predominant abnormalities occurred at the dermo–epidermal junction and were seen in sections stained for immunoglobulins and complement.

ACKNOWLEDGMENTS

We wish to thank Miss Hazel Cross and Mrs Janet O'Malley for excellent technical assistance and Mr R.A.Forster for the preparation of the photomicrographs.

This work was supported by grants from the Medical Research Council, Arthritis and Rheumatism Council and Leeds and West Riding Medical Research Trust.

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The deoxyribonucleic acid (DNA) skin test in systemic lupus erythematosus.

I. CLINICAL EVALUATION

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Accepted for publication 20 January 1975

SUMMARY

A clinical evaluation of the intradermal DNA-test was carried out on a series of patients with untreated or with treated definite systemic lupus erythematosus (SLE), or with suspected SLE with or without circulating antinuclear factors. A saline solution of a commercially available DNA-preparation was used. The course of the DNA reaction was followed for 24–48 h after the injection.

All nine cases of untreated definite SLE had a positive DNA test 6 h after the injection, and eight cases a positive result at 24 h. All seven patients with definite SLE who were on low-dosage systemic steroid treatment had a clinically positive DNA test at 6 h. In all except one of these cases the test was still positive at 24 h. All five patients with definite SLE on antimalarial treatment had a positive test at 6 h which had become negative at 24 h after the injection. Eleven of the twelve patients with suspected SLE and circulating antinuclear factors had a positive DNA test at 6 h, which in nine cases persisted for 24 h. On the other hand, of the eleven ANF negative patients with various connective tissue diseases five had a positive test at 6 h. In only one of these cases it persisted for 24 h. Two of the eighteen control patients with various dermatoses exhibited a positive test at 6 h, and in one of these the positive reaction persisted for 24 h.

It is concluded that the intradermal skin test using native DNA is a useful diagnostic tool for the detection of SLE when the reaction is followed up for 24 h. Antimalarial treatment seems to decrease skin reactivity to native DNA.

The first specific laboratory test for systemic lupus erythematosus (SLE), the LE-cell phenomenon (Hargraves, 1949), is based on phagocytosis of altered nuclear material. The nuclear antigen is related to deoxyribonucleic acid (DNA), as shown by Holman & Kunkel (1957) and Friou, Finch & Detre (1958). By immunofluorescence methods it has been found that patients with SLE often show high levels of humoral antinuclear antibodies. These are not entirely specific for SLE but have proved to be an important tool for screening purposes. On the other hand, antibodies against native DNA, although highly specific for SLE, have been found in only about 50% of cases with active SLE (Seligman, Cannat & Hamard, 1965).

Although skin lesions occur in 60–75% of patients with SLE (Tuffanelli, 1972), the role of skin reactivity to nuclear antigens has not been established. Some authors have demonstrated LE-cells in the skin (Perillie, Calabresi & Finch, 1960; Rebuck & Berman, 1950; Watson, O'Leary & Hargraves 1951; Wilson, Abbott & Miller, 1961). Further, some investigators have been able to demonstrate the LE cell phenomenon at the site of intradermal injection of leukocytes or DNA (Ghosh, 1972; Ores & Mandel, 1970; Rebuck & Berman, 1950), while others have failed to do so (Richter & Apostoloff, 1970).

Skin tests with DNA-material in patients with SLE have been reported by several authors but the results have been somewhat contradictory (Azoury *et al.*, 1966; Block *et al.*, 1968; Chieregato & Faldarini, 1964; Fardal & Winkelmann, 1965; Jones *et al.*, 1967; Ores & Lange, 1964). However, DNA from various sources has been used and its amount and degree of polymerization have varied. Further, the interpretations of the tests differ in these studies.

The present study is a clinical evaluation of the intradermal DNA-test in patients with untreated and with treated definite SLE, and in patients with suspected SLE with and without circulating antinuclear factors, as well as in controls.

MATERIALS AND METHODS

The material consisted of sixty-two patients hospitalized at the Department of Dermatology, University Central Hospital, Helsinki, during 1971–1973. A total of thirty-one patients were referred to the clinic for examination on account of suspected SLE. Ten of these patients had discoid lupus erythematosus (DLE). Thirteen of the sixty-two patients have been followed up for several years.

The control group contained eighteen patients. Nine were cases of acne, two of psoriasis and two of artefacts, the remaining cases being primary syphilis, contact eczema, lupus vulgaris, seborrhoeic dermatitis and an unknown rash.

The diagnosis of definite and probable SLE was based on the criteria previously proposed, including the same clinical and laboratory examinations (Johansson, 1971). In addition, a positive ANF test was required for the diagnosis of definite SLE. The ANF test was regarded as positive when the titre was 1:80 or higher. In all except one of the SLE cases the titre was 1:320 or higher and antinuclear antibodies were found in both IgG and IgM immunoglobulin classes. The ANF test was performed also in the control cases and found to be negative in all instances. The serum complement level was examined in patients but in none of the control cases.

Commercially available DNA preparations from the Mann Research Laboratory were used for the DNA test solutions. A highly polymerized calf thymus DNA (873) was used. The 0·1% test solution was prepared as initially described by Ores & Lange (1964). However, most of the tested patients complained initially of a painful sensation which lasted for several seconds after the injection. This was thought to be due at least partly to the fact that distilled water had been added to the solution. It was further suspected that the hypotonic solution as such might cause some reaction. When control tests were performed with the above mentioned solution (without DNA) and with a saline control solution, it was found that the initial reaction persisted longer when the former solution was used. The size of the induration did not differ but a marked erythema was recorded when the hypotonic solution was used. In two control cases tested with the hypotonic DNA-solution the reaction subsided first after 24 h. However, there were no histological changes in the biopsies taken at 8–10 or at 24 h (Johansson, Niemi & Lassus 1975).

Consequently a new 0·1% test solution was prepared, the DNA being directly dissolved in saline.

The test solution was rendered sterile by direct filtration into glass ampoules employing Millipore filter 0·45. The native state of the DNA was controlled from time to time according to the method described by Lin & Chargaff (1966). Subsequent periodical cultures revealed no growth of bacteria or fungi. Viral cultures were not performed.

A second DNA-test solution was prepared in the same manner as described above being made of DNA sodium salt (126) with a lower degree of polymerization than the DNA acid. The two solutions were used concurrently. However, this DNA sodium salt solution often gave negative results when the DNA acid solution gave positive results, and it was also negative in some of the patients with definite SLE. In addition there was no difference in specificity when the control patients were tested. The sodium salt of DNA was therefore not used later in the study and the results of the tests will not be evaluated.

For testing purposes 0·1 ml of the 0·1% solution was injected intradermally into the backs of the patients. During the first 6 h all tests were read every 1 h, and thereafter at 8–10 h, 24 h and 48 h. The test was regarded as positive when the size of the induration was 6 mm or more measured in two perpendicular directions. Erythema was often diffuse, and in size it was found to correlate with the size of the induration. It was therefore not taken into consideration. However, it must be mentioned that in all except control cases there was an erythema when the size of the reaction was 5 mm or over.

RESULTS

Clinical and serological data correlated with the DNA test result

The DNA test results of the sixty-two patients are presented in Table 1 as correlated with the age, sex and clinical diagnoses of the patients. At the time of testing a positive LE cell phenomenon was found in ten of the twenty-one patients with definite SLE. A total of sixteen SLE patients had a low serum complement level, while in five cases it was normal.

TABLE 1. DNA test results correlated with age and sex of the sixty-two patients in the different diagnostic groups

Clinical diagnosis and treatment groups	Patients							DNA test results at			
	Total	Sex		Age groups (years)				6 h		24 h	
		Female	Male	< 20	21–40	41–60	≥ 61	+	−	+	−
SLE definite; untreated	9	9	0		4	4	1	9	0	8	1
SLE definite; treated with steroids	7	6	1		6	1		7	0	6	1
SLE definite; treated with antimalarials	5	3	2	1	1	3		5	0	0	5
Connective tissue disease; ANF positive	12	11	1		3	5	4	11	1	9	3
Connective tissue disease; ANF negative	11	7	4	1	3	7		5	6	1	10
Controls	18	9	9	4	10	3	1	2	16	1	17
Total	62	45	17	6	27	23	6	39	23	25	37

TABLE 2. DNA test results correlated with some clinical and serological data of the twelve patients with a positive ANF test

Patient no.	Butterfly eruption	DLE	Photosensitivity	Urticaria	Drug eruption	Leg ulcers	Thrombosis	Fever, remittent, unexplained	Pleurisy	Nephropathy	Arthritis and/or arthralgia	CBFP	Leukopenia ≤3·0	ESR ≥70	ANF-titre	ANF-immunoglobulin class		Complement level in the serum		DNA test results			Clinical diagnoses
																IgG	IgM	C_3	C_4	6 h	24 h	48 h	
3	−	−	−	−	−	−	−	+	−	−	−	−	−	+	1280	320	20	n	↓	++	−	−	Non-definite connective tissue disease
5	−	−	−	+	−	−	−	−	−	+	−	−	−	+	1280	1280	5	n	n	+	++	−	Haemolytic anaemia
6	−	−	−			−	−		−	−	−	−	−	−	320	320	80	n	n	+	−	−	Erythema induratum Bazin*
8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	80	80	80	n	↓	+	+	+	Non-definite connective tissue disease
30	+	+	+	−	−	−	−	+	+	−	+	+	+	−	320	320	1280	n	↓	+	+	+	Probable SLE
31	−	−	+	+	−	−	−	+	−	−	+	−	−	+	1280	1280	1280	n	n	++	+	−	Probable SLE
32	−	−	+	+	−	−	−	+	−	−	+	+	+	−	80	20	20	↓	↓	−	+	−	Probable SLE
34	−	−	−	−	−	−	−	−	−	−	−	−	−	−	1280	1280	80	n	↓	+	−	−	Non-definite connective tissue disease
45	−	+	+	−	−	−	−	−	−	−	+	−	−	−	80	320	5	n	n	+	+	+	DLE
49	+	+	+	−	−	−	−	−	−	−	+	−	+	−	80	320	5	n	n	+	+	+	Probable SLE
50	−	+	+	−	−	−	−	−	−	−	−	−	+	−	80	—	20	n	n	+	++	−	Probable SLE
58	−	+	+	−	−	−	−	−	−	−	+	+	−	−	80	80	80	n	n	+	++	++	DLE†

Abbreviations: CBFP, Chronic biologic false positive sero-reactions for syphilis; n, normal; ↓, lowered.

* The patient was found to be ANF positive during treatment with isoniazid and PAS.

† The sister of this patient had definite SLE.

The clinical manifestations and some serological data of the twelve patients with a positive ANF test or with various diseases, mostly of autoimmune type, are presented in Table 2. There was a high degree of reactivity in the DNA test in this group.

Table 3 contains the same clinical data for the eleven patients with various connective tissue diseases showing a negative ANF test. Only one patient in this group had a positive DNA test which persisted for 48 h. This young man was the only patient with a lowered serum complement level in this group. There was a much lower frequency of reactivity to DNA in this group as compared with the group with positive ANF tests (Table 2).

TABLE 3. DNA test results correlated with some clinical data in the eleven patients with negative ANF test

Patient no.	Butterfly eruption	DLE	Photosensitivity	Urticaria	Drug eruption	Leg ulcers	Thrombosis	Fever, remittent	Pleurisy	Nephropathy	Arthritis and/or arthralgia	CBFP	Leukopenia ≤ 3·0	ESR ≥ 70	DNA test results			Clinical diagnoses
															6 h	24 h	48 h	
7	–	+	+	–	–	–	–	–	–	–	–	+	–	–	+	–	–	DLE
9	–	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	DLE
13	–	–	–	–	–	+	+	–	+	–	–	+	–	–	+	+	+	NDCD
22	–	+	–	–	–	–	–	–	–	–	+	–	–	–	++	–	–	DLE and RA
28	–	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	DLE
43	–	–	+	–	–	–	–	–	–	–	–	+	–	–	+	–	–	NDCD
51	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	NDCD
54	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	NDCD
55	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–	NDCD
56	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	NDCD
59	–	+	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–	DLE

Abbreviations: DLE, Discoid lupus erythematosus; RA, Rheumatoid arthritis; NDCD, Non-definite connective tissue disease.

Two of the control patients had a positive DNA test at 6 h and one of them still had a positive test at 24 h (Table 1). This was a patient with primary syphilis without evidence of connective tissue disease. He was one of the two who were tested with the hypotonic DNA solution and had a positive result with the control solution as well.

The course of the DNA reaction

In all positively reacting cases, reactivity was detectable within 6 h after the injection of the DNA solution. The reaction reached a peak at 24 h and subsided within 48–72 h after the injection. In Fig. 1 the course of the reaction in the different diagnostic groups is illustrated. The patients receiving systemic corticosteroid therapy were having doses varying from 5 to 30 mg of prednisone per day and the patients on antimalarial treatment received 100 mg of chloroquine or hydroxychloroquine per day. As is seen from Fig. 1 the patients with definite SLE on corticosteroid treatment had a DNA test result very similar to that of the cases of untreated SLE, while the reaction subsided faster in the cases on antimalarial treatment. Only one of the patients with connective tissue disease and a positive ANF test was on corticosteroid therapy and none except the five patients with definite SLE received antimalarial therapy.

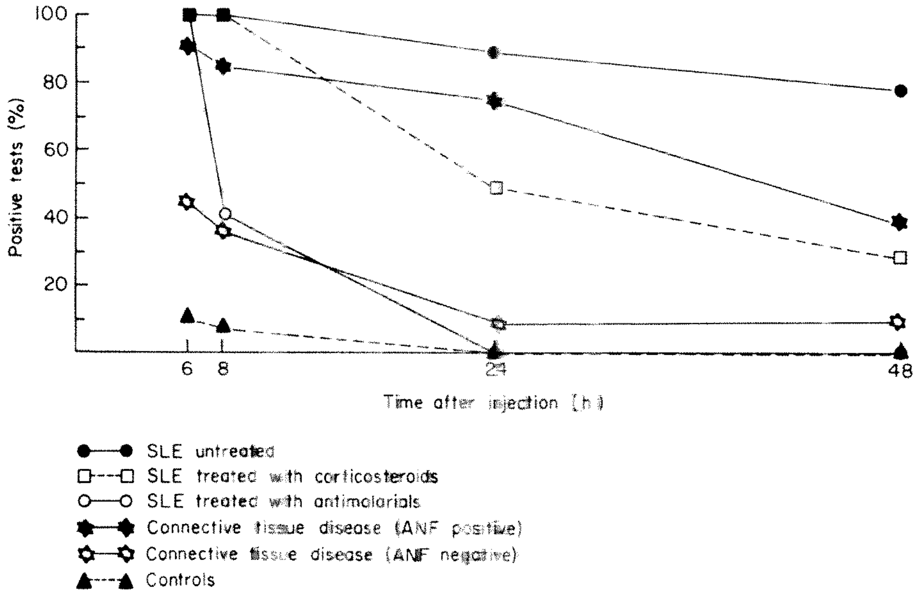

FIGURE 1. The course of the DNA reaction in different diagnostic and treatment groups.

DISCUSSION

Calf-thymus DNA has been used as intradermal test-material in several previous studies. In some of these an increased skin reactivity to DNA has been shown in cases with SLE (Azoury *et al.*, 1966; Fardal & Winkelmann, 1965; Ores & Lange, 1964; Ores & Mandel, 1970). In other studies (Block *et al.*, 1968; Chieregato & Faldarini, 1964) no increased sensitivity has been found in SLE as compared with controls or patients with other connective tissue diseases. The contradictory results are probably due to differences in technique and in activity of the disease. Antimalarial drugs in particular are known to interact with nucleic acids and nucleoproteins (Parker & Irvin, 1952). Actually, as shown by Levin & Pinkus (1961), autosensitive inflammatory skin lesions due to DNA can be controlled by chloroquine. High doses of corticosteroids also seem to inhibit the skin reaction to DNA in SLE (Azoury *et al.*, 1966). The state of the DNA is also essential since, as shown by Arana & Seligmann (1967), SLE patients may have antibodies to native DNA solely, or to denaturated DNA solely, or to both forms. Tan and Natali (1970) showed that there is no cross-reaction between the two forms of DNA. Normally, in contrast to denaturated DNA, against which antibodies have been found in a variety of disorders (Koffler *et al.*, 1969), there is no immune response to native DNA.

In the present study a solution of native DNA was used. Our results suggest that the intradermal DNA test using this antigen is a reliable tool for detecting active SLE. The reactions should be read for as long as 24 h since it seems that the reactivity is much more prolonged in SLE than in other connective tissue diseases. The skin reactivity of the SLE patients on antimalarial treatment was reduced, but low doses of prednisone did not seem to interfere significantly with the test results. On the other hand, corticosteroid treatment does seem to interfere with humoral DNA-antibodies (Richter & Apostoloff, 1970). As shown by Pincus *et al.* (1969), measuring the ability of the serum to bind DNA seems to be a more sensitive method of detecting the latter.

It seems justified to conclude that the intradermal skin test using native DNA is a useful diagnostic tool for the detection of SLE when the test results are followed up for at least 24 h.

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The deoxyribonucleic acid (DNA) skin test in systemic lupus erythematosus.

2. HISTOLOGICAL FINDINGS

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Accepted for publication 20 January 1975

SUMMARY

A total of fifty-five biopsies from fifty-two intradermal DNA skin tests were studied. The biopsies were taken 6, 8–10, 24 or 48 h after the injection of the DNA material. Necrosis of the vessel wall was taken to be the main characteristic of a specific reaction. In forty of the fifty-two tests the results of the histological evaluation closely matched the clinical results. In five of the fifteen cases with discrepancies, the histological evaluation ruled out clinically false positive test results.

In three cases of SLE on corticosteroid treatment, the histological examination gave a positive result despite a clinically negative result.

In seven of the fifteen cases the discrepancies occurred in borderline cases with reactions of 5 to 6 mm diameter.

The amount of inflammatory cells in positive as well as in negative reactions was also recorded. The number of polymorphonuclear cells in positive reactions increased with the age of the reaction. The number of lymphocytes was not found to increase in the positive reactions, thus differing from the delayed hypersensitivity type of reactions. Rather, the reaction was characterized by an Arthus type of hypersensitivity.

On the basis of the present study it may be concluded that clinically positive tests at 6 or 8 h may merely be expressions of nonspecific vascular alterations. On the other hand, in late reactions, even in patients on systemic treatment, histological examination revealed clinically negative results to be positive. By using the histological picture of hypersensitivity angiitis as the main diagnostic criterion the specificity of the clinical reactions may be established.

In a previous study (Johansson *et al.*, 1975) we found the DNA skin test to be valuable for the diagnosis of systemic lupus erythematosus (SLE). When comparisons were made with other connective tissue diseases the reactivity was found to be much more prolonged in SLE. Consequently it was suggested that the DNA-reaction should be followed up for at least 24 h.

The purpose of the present study is to establish the histological criteria for a positive DNA-test and to study the evolution of the tissue reaction over a period of 48 h in order to ascertain the specificity of the test.

MATERIALS AND METHODS

The series comprised thirty-nine patients. They all belonged to the group of sixty-two patients presented in the first paper (Johansson *et al.*, 1975). Eight of the thirty-nine were dermato-venereological patients without evidence of connective tissue disease. The DNA skin tests were performed as described earlier. Punch biopsies were taken from fifty-five intradermal skin tests 6, 8–10, 24 or 48 h after the injection of the test material.

In all instances the histological evaluation was carried out without knowledge of the patient or the size or nature of the DNA-reaction. The biopsy specimens were processed in paraffin, cut and stained by haematoxylin–eosin and Unna–Pappenheim methods. The number of inflammatory cells was tabulated and special attention was paid to the condition of blood vessels.

RESULTS

Necrosis of the vessel wall was the main characteristic of a specific reaction. Necrosis could be seen as disappearance of the endothelial cells or as fibrinoid degeneration of the vessel wall (Fig. 1), mostly accompanied by infiltration of neutrophils or red cells into the vessel wall. Vacuolization or oedema of the vessel wall were not considered to be a specific sign of a positive reaction.

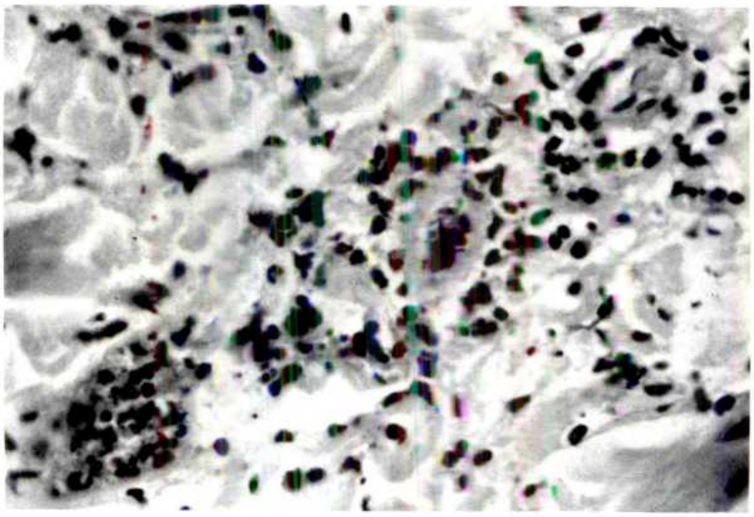

FIGURE 1. Clinically a borderline test 5 mm in diameter (= negative) showed, after 24 h, a picture of vasculitis with polymorphonuclear leukocytes and nuclear dust and fibrinoid degeneration of the vascular wall. The patient was ANF positive, suspected SLE (H & E., 12.5 × 10).

In forty of the fifty-five biopsies the results of the histological examination closely matched the clinical test results (Table 1). Four of the fifteen cases in which discrepant results were obtained were control test cases, two being from control patients and two being tests performed with the control solution. In these cases the histological examination ruled out clinically false-positive test results.

A total of twenty-one biopsies from SLE patients were studied. In two cases of untreated and five

TABLE 1. The DNA test results correlated with the results of the histological examination in the various diagnostic groups

Time after biopsy (h)	Clinical test results	SLE untreated: histologically		SLE treated: histologically		NDCT ANF+: histologically		NDCT ANF: histologically		Controls: histologically	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
6	Positive	3*	—	5	1†	4	2‡	1	—	—	—
	Negative	—	—	—	—	—	1‡	—	1	—	2
8–10	Positive	1	—	—	—	—	2	—	1	—	2
	Negative	—	—	1	—	—	—	—	2	—	4
24	Positive	1	—	—	1†	2	—	—	—	—	—
	Negative	—	—	1	1	1	4	—	2	—	2
48	Positive	1	1	2	—	—	—	1	—	—	—
	Negative	1*	1	1	1	—	—	—	—	—	—
No. of biopsies in each group		21				16		8		10	

NDCD, nondefinite connective tissue disease.

* The same patient, † the same patient, ‡ the same patient.

cases of treated SLE discrepant results were obtained in the histological and clinical evaluations. In four of these (three were under treatment) the histological examination revealed a positive result despite a clinically negative one. In three cases of SLE, one of which was untreated, the histological examination did not confirm a borderline clinical positive result (reaction of 6 mm in diameter).

Sixteen biopsies were taken from patients having a non-definite connective tissue disease with a positive antinuclear factor ANF-test. In two of these cases a borderline clinical result was not histologically confirmed. A third patient with a mixed type of connective tissue disease and a low serum complement level had a negative clinical test. Nevertheless, the histological evaluation revealed a little surface erosion and necrosis of the capillaries, which was considered positive.

In the eight biopsies from patients with non-definite connective tissue disease and a negative ANF-test, only one discrepant result was obtained. The patient was a healthy young woman with a CBFP-reaction, who at 8 h had a borderline clinically positive DNA test which was histologically negative.

The fact that discrepancies mostly occurred in the clinically borderline cases is further illustrated in Table 2. The majority of the cases which were clinically positive but histologically negative occurred in the group with reactions 6–9 mm in diameter.

TABLE 2. The degree of DNA-reactivity correlated with the results of the histological examination

DNA-test results graded	Positive and negative histological findings at:							
	6 h		8–10 h		24 h		48 h	
	+	−	+	−	+	−	+	−
+ + +	1						1	
+ +	4		1	2			2	
+	6	3		3	3	1	1	1
−		4	1	6	2	9	2	2

+ + +, induration ≥15 mm; + +, induration 10–15 mm; +, induration 6–9 mm. −; induration ≤5 mm.

The numbers of inflammatory cells in biopsies in histologically positive as well as negative cases are shown in Table 3. The number of polymorphonuclear leukocytes in positive reactions increased with the age of the reaction (Fig. 2a, b).

The amount of lymphocytes was small in all cases and they were almost absent in some of the specimens. In histologically negative cases their number increased slightly with the age of the reaction; whereas in positive cases, in distinction from the polymorphonuclears, there was no increase.

The amount of eosinophils was also small in all cases, and their number did not increase with the age of the reaction.

Special attention was paid to the occurrence of plasma cells counted in specimens stained by the Unna–Pappenheim method. The number of plasma cells was not found to be increased when compared with the number of other cells present. Neither were there differences between histologically positive and histologically negative reactions. The number of plasma cells did not increase with the age of the reaction. Neither LE-cells nor haematoxylin bodies were observed in any of the histologically positive cases.

TABLE 3. The amount of inflammatory cells in histologically positive and negative reactions

Time (h)	Positive reaction											
	polymorphonuclear			eosinophils			plasma cells			lymphocytes		
	−	+	++	−	+	++	−	+	++	−	+	++
6	−	6	5	3	6	2	3	6	2	2	7	2
8–10	−	−	2	2	−	−	2	−	−	1	1	−
24	−	1	4	4	1	−	−	3	2	1	4	−
48	−	1	5	3	3	−	2	4	−	1	3	2
	Negative reaction											
6	1	3	3	4	3	−	2	3	2	5	2	−
8–10	2	7	2	9	2	−	5	5	1	−	7	4
24	2	4	4	8	2	−	5	4	1	3	5	2
48	1	2	−	3	−	−	2	−	1	−	1	2

−, practically no cells; +, moderate or few cells; ++, considerable amount of cells.

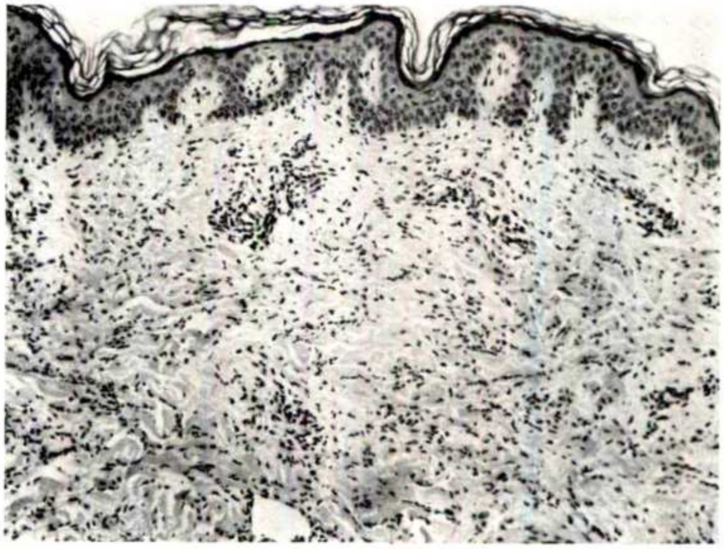

FIGURE 2(a). The patient had untreated SLE. Clinically and histologically positive test after 6 h. There is a large number of polymorphonuclear leukocytes diffusely in the tissue (H & E., 12·5 × 2·5).

DISCUSSION

Histological examination of DNA skin tests has been performed in few investigations, on limited numbers of cases and without consistent results. Ores & Mandel (1970) used DNA material in three

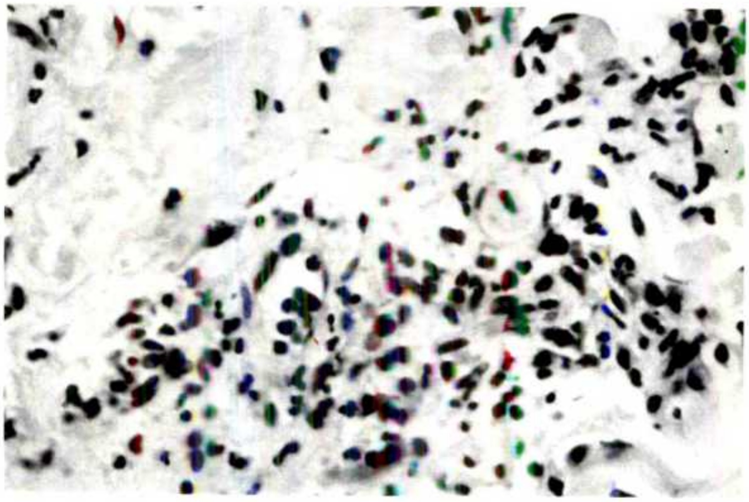

FIGURE 2(b). Higher magnification of the same picture as 2(a). The vascular necrosis is already complete (H & E, 12·5 × 10).

cases of SLE and stated that the response was an immediate type of hypersensitivity 8–12 h after the injection and a delayed type of hypersensitivity 24 h after the injection. They also found LE-cells at the site of injection. Fardal & Winkelmann (1965) carried out a histological study in three DNA-tested cases. They found a perivascular infiltration of mononuclear cells without necrosis of the vessel wall in three biopsies taken at 20 h.

Bennet & Holley (1961), who used normal leukocytes instead of DNA in the tests, concluded that a positive reaction represented both Arthus and delayed type of hypersensitivity.

In the present study the results of the histological examination confirmed the results of the clinical evaluation in the cases of untreated SLE. However, patients in remission due to antimalarial or corticosteroid treatment showed discrepancies, especially those on corticosteroid treatment. Nevertheless, according to the present study the histological evaluation might indicate a positive DNA skin test in some cases despite a clinically negative test. The study also shows that a histological examination often rules out any clinically false-positive reactions.

The histological examination of the DNA-reactions also revealed that clinically positive tests at 6–10 h might merely be expressions of nonspecific vascular alterations. On the other hand, if the histological picture of hypersensitivity angiitis is used as the main diagnostic criterion, the specificity of the clinical reactions may be established. Further, in late reactions, even in patients on systemic treatment, the histological examination revealed clinically negative results to be positive. It seems that the optimal time for biopsy is between 24 and 48 h after the DNA challenge.

The cellular response was by no means specific. According to Ores & Mandel (1970) polymorphonuclear leukocytes are found in early reactions and mononuclear cells predominate in late reactions. This contradicts our findings; but their results were based on a few histological specimens only.

Speculations on the nature of the hypersensitivity reaction are based on histological findings. The vascular damage seen regularly in these tests is a hallmark of the Arthus type of reaction (Turk, 1969). However, the prolonged duration is unusual, owing perhaps to the comparatively large amount of antigen material. Additional factors are its high viscosity and the slow release of antigenic determinants at the injection sites.

The small quantity of lymphocytic infiltration speaks against a delayed type of reaction (Parish, 1970; Spector, 1967). The fact that no increase was observed in the number of plasma cells in the late reactions is also of interest. In experimental studies it has been shown that plasma cells appear at the site of antigen–antibody reaction after antigen challenge (Turk, 1967) when circulating antibodies are present.

Native DNA is known to be a poor immunogen. However, renal damage in SLE is assumed to be caused by a soluble complex of DNA with anti-DNA antibody and complement (Tuffanelli, 1972). The histological study of the present series suggests that the same type of reaction may play the main role in DNA test sites on the skin.

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British Journal of Dermatology (1975) **92**, 459.

An improved method of assessing topical corticosteroid activity

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Accepted for publication 26 July 1974

SUMMARY

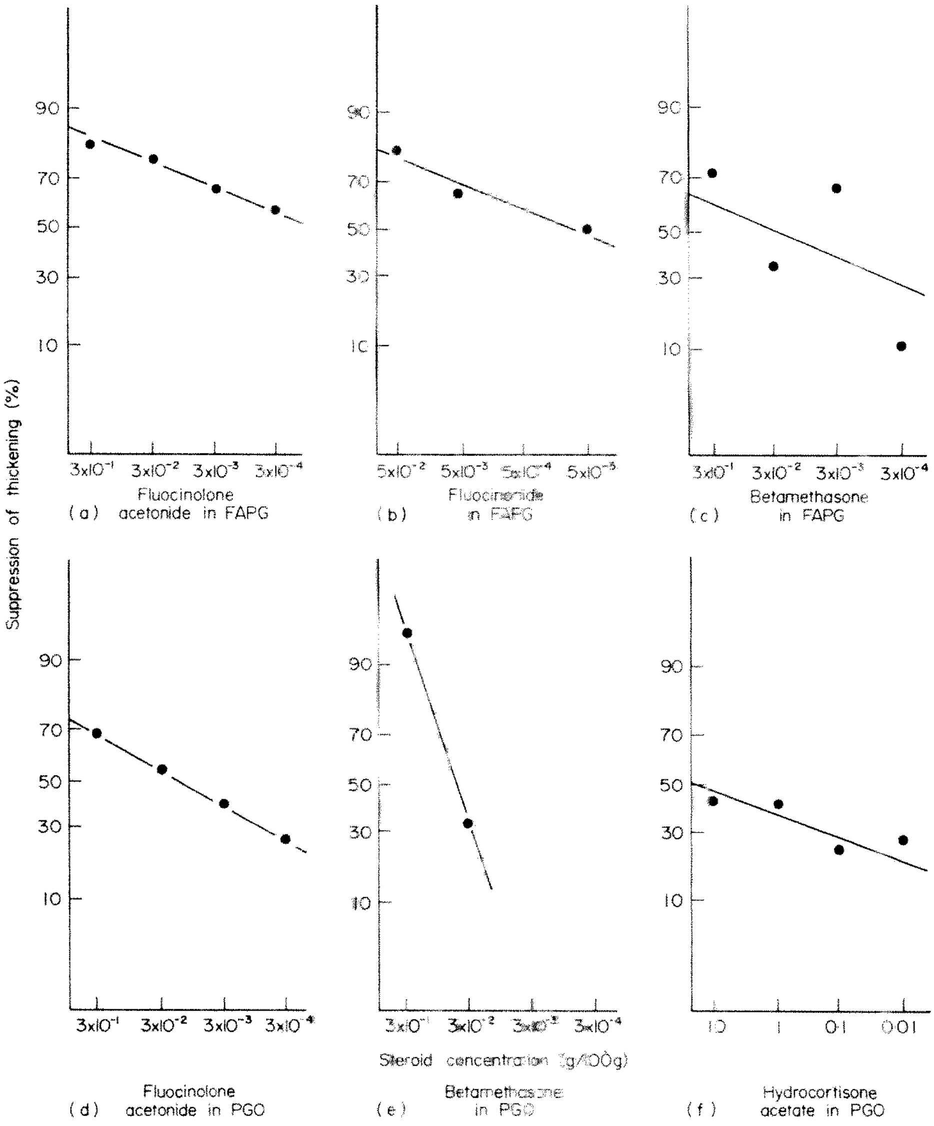
Topical application of ointment bases causes varying degrees of epidermal thickening in guinea-pigs This is reproducible and can be accurately measured. Suppression of these changes was produced by addition of beta-methasone 17-valerate, fluocinolone acetonide, fluocinonide and hydrocortisone acetate. The inhibitory effect of these steroids was related to the type of corticosteroid, its concentration and the ointment base. The effect was still evident at extremely high dilutions of the steroids and could be measured at concentrations as low as $5 \times 10^{-5}\%$ of fluocinonide in FAPG.

This technique has the merits of being reproducible, sensitive and accurate. It should find a place among existing methods in assaying the efficacy of topical corticosteroids and in aiding the selection of bases most suitable for formulation of these preparations.

Available techniques for measuring topical corticosteroid effects include the use of artificial and natural membranes *in vitro*, animal experiments, investigative and clinical assessment in man. *In vitro* studies, on the whole, are useful in screening new products but more reliable assessment can be made by the use of *in vivo* tests. Although animal experiments cannot be applied without reservations to human conditions, these may provide more accurate data which can subsequently be confirmed in human trials. Existing tests either measure absorption of the applied steroid by radiochemical techniques or they are based on measurement of physiological changes produced by the steroid in the skin, i.e. blanching or suppression of erythema. With the exception of studying the effect of steroids on mitotic activity in the epidermis *in vivo*, all methods are based on indirect effects which follow absorption of the active agent through the skin. They may not reflect directly the anti-inflammatory action of the steroid.

We have devised a new method of assessing topical corticosteroid activity based on objective criteria. We suggest that it will be of use both in comparing the effects of available preparations and investigating new ones.

Certain ointment bases applied to guinea-pig skin produce measurable and reproducible epidermal thickening. (Sarkany & Gaylarde, 1973; Schaaf & Gross, 1953.) It has been shown previously that skin thickness in animals increases in response to allergens or non-specific irritants, such as old tuberculin, phytohaemagglutinin, iodine, formalin, phenol, xylene and benzene (Sarkany & Fountain,



(a) Fluocinolone acetonide in FAPG
(b) Fluocinonide in FAPG
(c) Betamethasone in FAPG
(d) Fluocinolone acetonide in PGO
(e) Betamethasone in PGO
(f) Hydrocortisone acetate in PGO

FIGURE 1. (a) Thickening induced by FAPG suppressed by fluocinolone acetonide; (b) Thickening induced by FAPG suppressed by fluocinonide; (c) Thickening induced by FAPG suppressed by betamethasone 17-valerate; (d) Thickening induced by PGO suppressed by fluocinolone acetonide (e) Thickening induced by PGO suppressed by betamethasone 17-valerate; (f) Thickening induced by PGO suppressed by hydrocortisone acetate.

1967). Addition of steroids in various concentrations to the bases reduces the thickening caused by the ointment base alone. These effects form the basis of the technique described in this paper. The method has been found to be more accurate than other available experimental techniques and more sensitive than clinically measurable responses produced by topical steroids in humans.

MATERIALS AND METHODS

Six groups of albino guinea-pigs were used. The backs were clipped and the area divided into six equal parts. One site was used as a control, the ointment base was applied to another site and four different concentrations of steroid in the ointment base were rubbed into the remaining sites daily for 4 or 7 days. The animals were killed on the 5th or 8th day, the hair was removed with barium sulphide, the treated areas and control sites were fixed in formol saline and processed for histology. Sections of 5 μm were cut and stained with haematoxylin and eosin. Epidermal thickness was measured at twenty points of each specimen, using an eye piece graticule. Each graticule division represents 2·42 μm. The steroids used in the study were fluocinonide in fatty alcohol propylene glycol (FAPG), flucinolone acetonide in FAPG and in propylene glycol ointment (PGO), betamethasone 17-valerate in FAPG and in propylene glycol, and hydrocortisone acetate in propylene glycol ointment. The FAPG and PGO were kindly supplied by I.C.I.

RESULTS

The results (Fig. 1, Tables 1–6) showed that the ointment bases used caused epidermal thickening and that the addition of steroids reduced this change. The effect of steroids varied with different

TABLE 1. Average thickness of the epidermis (in graticule divisions) following treatment with fluocinolone acetonide in FAPG. n in all tables represents number of animals used

Treatment	Thickness (graticule divisions)		Increase over control	Increase over control (%) (ointment base = 100%)
	Mean	s.e. (n = 8)		
Control	8·53	0·72	0	0
3×10^{-1}% Fluocinolone acetonide in FAPG	10·63	1·28	2·10	18·3
3×10^{-2}% Fluocinolone acetonide in FAPG	11·15	1·17	2·62	22·8
3×10^{-3}% Fluocinolone acetonide in FAPG	12·53	2·29	4·00	34·9
3×10^{-4}% Fluocinolone acetonide in FAPG	13·57	1·25	5·04	43·9
FAPG	20·00	1·62	11·47	100

TABLE 2. Average thickness of the epidermis (in graticule divisions) following treatment with fluocinonide in FAPG

Treatment	Thickness (graticule divisions)		Increase over control	Increase over control (%) (ointment base = 100%)
	Mean	s.e. (n = 6)		
Control	7·59	0·23	0	0
5×10^{-2}% Fluocinonide in FAPG	9·90	0·70	2·31	19·4
5×10^{-3}% Fluocinonide in FAPG	11·62	1·54	4·03	33·8
5×10^{-4}% Fluocinonide in FAPG	19·84	2·37	12·25	—
5×10^{-5}% Fluocinonide in FAPG	13·53	1·45	5·94	49·7
FAPG	19·51	1·60	11·92	100

TABLE 3. Average thickness of the epidermis (in graticule divisions) following treatment with betamethasone 17-valerate in FAPG

Treatment	Thickness (graticule divisions)		Increase over control	Increase over control (%) (ointment base = 100%)
	Mean	s.e. (n = 5)		
Control	8·99	0·58	0	0
3×10^{-1}% Betamethasone 17-valerate in FAPG	10·48	0·85	1·49	26·3
3×10^{-2}% Betamethasone 17-valerate in FAPG	12·76	1·61	3·77	66·1
3×10^{-3}% Betamethasone 17-valerate in FAPG	10·89	0·91	1·90	33·6
3×10^{-4}% Betamethasone 17-valerate in FAPG	14·1	0·81	5·11	90·4
FAPG	14·64	2·23	5·65	100

TABLE 4. Average thickness of the epidermis (in graticule divisions) following treatment with fluocinolone acetonide in PGO

Treatment	Thickness (graticule divisions)		Increase over control	Increase over control (%) (ointment base = 100%)
	Mean	s.e. (n = 6)		
Control	11·20	0·93	0	0
3×10^{-1}% Fluocinolone acetonide in PGO	13·36	1·57	2·16	27·8
3×10^{-2}% Fluocinolone acetonide in PGO	14·54	1·55	3·34	43·0
3×10^{-3}% Fluocinolone acetonide in PGO	15·82	1·90	4·62	59·5
3×10^{-4}% Fluocinolone acetonide in PGO	16·79	1·50	5·59	71·9
PGO	18·97	1·52	7·77	100

TABLE 5. Average thickness of the epidermis (in graticule divisions) following treatment with betamethasone 17-valerate in PGO

Treatment	Thickness (graticule divisions)		Increase over control	Increase over control (%) (ointment base = 100%)
	Mean	s.e. (n = 6)		
Control	10·84	0·62	0	0
3×10^{-1}% Betamethasone 17-valerate in PGO	10·94	0·82	0·10	3·8
3×10^{-2}% Betamethasone 17-valerate in PGO	12·64	0·92	1·79	68·0
3×10^{-3}% Betamethasone 17-valerate in PGO	14·01	0·77	3·16	—
3×10^{-4}% Betamethasone 17-valerate in PGO	13·82	0·73	2·97	—
PGO	13·48	0·79	2·63	100

TABLE 6. Average thickness of the epidermis (in graticule divisions) following treatment with hydrocortisone acetate in PGO

Treatment	Thickness (graticule divisions)		Increase over control	Increase over control (%) (ointment base = 100%)
	Mean	s.e. (n = 6)		
Control	9·85	0·85	0	0
10% Hydrocortisone acetate in PGO	13·91	1·28	4·06	57·0
1% Hydrocortisone acetate in PGO	14·01	0·95	4·16	58·5
10^{-10}% Hydrocortisone acetate in PGO	15·33	1·75	5·48	77·1
10^{-20}% Hydrocortisone acetate in PGO	15·04	1·45	5·19	73·0
PGO	16·96	1·41	7·11	100

preparations and was dependent on the concentration of the steroid. Fig. 1a, b show that fluocinolone acetonide in FAPG and fluocinonide in FAPG have a similar degree of activity. These two combinations of steroid and base were the most effective. Hydrocortisone acetate in propylene glycol ointment caused no significant reduction of epidermal thickening after 4 days treatment and the remaining three preparations produced an intermediate degree of suppression of skin thickening. However, after more prolonged application of hydrocortisone acetate in propylene glycol ointment (7 days), similar changes of skin thickening to those seen with the other corticosteroids in 4 days, were observed (Fig. 1f, Table 6).

DISCUSSION

This work shows that measurement of changes in skin thickness in the guinea-pig is an accurate index of the irritant effects of various topical applications. Inhibition of these changes can also be reliably quantified. The three fluorinated corticosteroids in different vehicles produced inhibitory effects in concentrations as low 5×10^{-5}%, i.e. at a level several orders of magnitude lower than those used clinically. That this finding may be of practical value is supported by clinical experience which has led to widespread use of diluted topical steroids. Blank (1961) showed that 0·01% triamcinolone acetonide was only marginally less effective than the standard 0·1% preparation and others have made similar observations (Cahn & Levy, 1959; Witten, Sulzberger & Arthur, 1960). Vasoconstriction studies with topical steroids have also demonstrated the activity of these in very dilute concentrations and they too have shown similar logarithmic type dose–response curves (McKenzie, 1962).

Hydrocortisone acetate in propylene glycol behaved differently than the fluorinated steroids in requiring more prolonged application to the guinea-pig skin for the demonstration of similar changes. No definite changes were present after 4 days and statistically reliable results were obtained only after 7 days of treatment. Changes in the ointment base altered the degree of inhibitory activity of the corticosteroids used in this study. The technique described in this paper should, therefore, prove useful in helping to select the most suitable vehicle for a particular steroid and it should play a part in formulation of new preparations.

ACKNOWLEDGMENTS

We are grateful to the Peter Samuel Royal Free Fund and the Dunhill Trust for financial support.

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British Journal of Dermatology (1975) **93**, 465.

Case Report

Lichen planus and ulcerative colitis

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Accepted for publication 24 January 1975

SUMMARY

Three patients with ulcerative colitis and lichen planus are reported and similar cases in the literature discussed. Similarities in the pathology of the two conditions are outlined.

Ulcerative colitis patients have been seen who developed a lichenoid eruption attributed to salazopyrine (Clarke, 1972, 1974). A patient with ulcerative colitis and a lichenoid eruption was described whose rash got worse after withdrawal of salazopyrine (Wilson, 1973). A further patient with ulcerative colitis, lichen planus and myasthenia gravis is on record, together with extensive immunological studies (Miller, 1971). In each of the following cases of ulcerative colitis, salazopyrine appears not to have been involved in the genesis of an eruption closely resembling classical lichen planus.

CASE REPORTS

1. *J.E.* male, aged 36 years, a chicken farmer, began to complain of diarrhoea at the end of 1969. At approximately the same time and before any therapy was given he noticed a non-pruritic rash occurring patchily on different parts of the body, particularly prominent on the medial aspects of his forearms. Sigmoidoscopy showed pus, contact bleeding and a variable vascular pattern. Ulcerative colitis was demonstrated radiographically in the distal part of the transverse colon and in the whole of the descending colon. His haemoglobin was 11·9 g % with a white blood cell count of 6000/mm^3, the polymorphs showing toxic granulation. Serum mucoprotein was 354 mg/100 ml. He was treated with salazopyrine, methyl cellulose and prednisolone enemas with reasonable control of his bowel symptoms. However, the eruption became gradually more prominent. When referred for dermatological opinion in April 1972, multiple purple polygonal papules with Wickham's striae were present on his forearms and mid-back. A few mouth lesions of lichen planus were seen. Skin histology was non-specific. A good response was obtained to topical fluocinolone acetonide 0·2% cream. He shortly moved to a different district and has been lost to follow up.

2. *W.R.* male, aged 45 years, a police inspector, developed a blistering, itchy eruption on the hands and wrists in June 1973. Three days later he began to get diarrhoea with blood, mucus and abdominal pain. Sigmoidoscopy showed a granular and friable mucosa. Rectal histology showed an increased number of chronic inflammatory cells in the lamina propria, a few glands containing polymorpho-

nuclear exudate in their lumina. Barium enema showed a redundant loop of sigmoid colon and one area of the rectum did not distend well but there was no definite mucosal abnormality. The haemoglobin was 15·9 g% and there was a white blood cell count of 12,000/mm^3. The serum mucoprotein was 168 mg/100 ml. He responded to prednisolone enemas and methyl cellulose. Since then he has had intermittent diarrhoea. Further sigmoidoscopy in January 1974 showed a granular mucosa but no definite inflammatory change. Two months after the onset of the bowel trouble a dermatological opinion was requested and he showed widespread classical lichen planus of his limbs and trunk with no evident mucous membrane involvement. Many of the lesions on the lower limbs were bullous. Skin histology showed: 'a little hyperkeratosis and epidermal hyperplasia. There is some splitting of the epidermis from the dermis with the formation of a subepidermal bulla. In the upper dermis there is a band-like infiltration of inflammatory cells composed mainly of lymphocytes and polymorphs. The picture is suggestive of bullous lichen planus.' Treatment was carried out with fluclorolone acetonide 0·025% cream under polythene cover, a gradual improvement occurred, and 1 year later the eruption had settled.

3. *J.McQ.* female, aged 51 years, a housewife, started to have diarrhoea in January 1970. Sigmoidoscopy in February 1970 was normal but a barium enema in March 1970 showed a loss of haustration. The haemoglobin was 13 g% with a white cell count of 6000/mm^3 and an E.S.R. of 45 mm/h. The serum mucoprotein was 177 mg/100 ml. She was treated with a short course of systemic steroids and oral salazopyrine. On this regime her symptoms gradually settled down and when diarrhoea occurred, it was easily controlled with methyl cellulose, codeine phosphate and alginates. By the spring of 1971 she had formed stools and was off specific therapy. A repeat barium enema in November 1971 showed definite colitis of the descending and sigmoid colon. In January 1972 the diarrhoea became more prominent. Sigmoidoscopy showed a general slight abnormality with a red granular mucosa. In some places the vessel pattern was visible but there was no friability on swabbing. Rectal biopsy showed surface ulceration and considerable chronic inflammatory change extending into the muscle. The submucosa was oedematous and showed some fibrosis. No biochemical evidence of malabsorption was found. Diarrhoea recurred in January 1974, a barium enema showing further loss of haustration and of mucosal pattern indicating progress of ulcerative colitis.

In May 1973 she began to get an irritable skin eruption which started on the wrists and then spread to the feet. It was 2 years since she had taken salazopyrine. In view of further spread she was referred for dermatological opinion in September 1973 when she showed classical lichen planus of the trunk, limbs, lips and mouth. Skin histology showed: 'The epidermis shows fairly marked hyperkeratosis, a prominent stratum granulosum, acanthosis, and elongation of the rete ridges several of which show saw toothing. In some areas there is degeneration of the basal layer. A fairly dense infiltrate which consists mainly of lymphocytes is present in the upper dermis.'

Topical treatment with flucortolone acetonide 0·025% cream, partly under polythene cover, was associated with gradual fading of the rash over the following 15 months.

DISCUSSION

Ulcerative colitis and lichen planus are both diseases of unknown aetiology in which immunological processes play a part. Neither is a rare disease; the concurrence of the two conditions in the same patient might sometimes be expected by chance. Simultaneous onset of gut and skin symptoms in two of our patients poses the question of a common factor.

Lichen planus frequently affects the oral mucous membrane and sometimes the genital mucous membrane. There are single case reports of involvement of the stomach (Milian & Périn, 1936) and the bladder (Young, 1940) but the evidence for the diagnosis was in both cases circumstantial. No firm evidence exists therefore for recognizing lichen planus in structures of endodermal origin. Johnson & Wilson (1969) reviewed 415 ulcerative colitis patients and found twenty of them to have skin conditions thought to be associated with the underlying bowel disease. Several patients had more than one type of cutaneous lesion. Eight women had erythema nodosum appearing with exacerbations of the colitis; seven patients had pyoderma gangrenosum, apparently unrelated to the severity of the colitis; five patients developed small erythematous papules which broke down to form ulcers; three patients had ulcerating erythematous plaques on the shins. There were no cases of lichen planus (Wilson, 1974).

Abnormal glucose tolerance has been confirmed in lichen planus, suggesting a widespread metabolic disturbance (Powell *et al.*, 1974). In lichen planus an erosive process affects generative basal cells which are continuously replaced by the migration of epidermal cells from nearby (Marks, Black & Wilson Jones, 1973). Colloid bodies are formed by fibrillar transformation of basal cells; a band-like upper dermal infiltrate consists mostly of lymphocytes. Complement, fibrin and immunoglobulins IgG, IgM and IgA are demonstrated in this zone (Baart de la Faille-Kuyper & Baart de la Faille, 1974). A review of the aetiology of lichen planus recently appeared in the *British Medical Journal* (1974).

Lumb & Prothero (1957) suggested an intrinsic defect in epithelial regeneration in ulcerative colitis. The earliest lesion would appear to be an inflammatory process in the mucosal crypt where epithelial cells form vacuoles and die, leaving tiny crypt ulcers (Sleisenger & Fordtran, 1973). At first polymorphs are associated with the degenerative epithelial cells but later a chronic inflammatory infiltrate develops consisting of small round cells with a mixture of eosinophils and mast cells. In ulcerative colitis the continuing inflammatory process in the mucosa and submucosa is associated with a thirty-fold increase in mucosal IgG immunocytes, whose function appears to mediate continuing mucosal damage, although IgA and IgM production and transport occur normally (Brandtzaeg *et al.*, 1974). Jewell (1973) has reviewed recent work on the aetiology of ulcerative colitis.

To three previously described cases (Miller, 1971; Clarke, 1972; Wilson, 1973) of lichen planus and ulcerative colitis we add three personal cases of the concurrence of the two diseases, in each of which the suggestion of drug induced skin disease due to therapy of the colitis seems to be ruled out. In two of our cases the rash started at the same time as the first bowel symptoms. There are resemblances between the pathology of these two diseases in both of which it appears that epithelial cell turnover may be diminished and individual cell death (apoptosis) occurs followed by a small round cell infiltrate closely applied to the basement membrane, with immunological disturbance. The skin and gut changes in these patients could therefore have a causal rather than a fortuitous relationship.

ACKNOWLEDGMENTS

Thanks are due to Dr John Bennett who referred the patients and to Dr Janet Marks for reading the manuscript.

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British Journal of Dermatology (1975) **93**, 469.

Brief communications

Membrane-bound ATP-hydrolytic activity of psoriatic epidermis

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Accepted for publication 24 April 1975

Using electron microscopy histochemical techniques, Mahrle & Orfanos (1974) have recently reported that 'in contrast to normal epidermis, only minimal ATP-hydrolytic activity is present on the cell membranes of psoriatic keratinocytes'. We have attempted to throw further light on this important observation by quantitative biochemical measurement of membrane-bound ATPase activity.

Epidermal slices (0·2 mm thickness) were removed using a keratotome, and were homogenized in bovine serum albumin solution (1 mg/ml) containing 1 mmol/l EDTA. The homogenate was centrifuged for 10 min at 2400 *g*. The residue was washed and finally resuspended in the same medium at a concentration of 20 mg/ml. Incubation mixtures were as follows:

Mg^{++} activated ATPase. 4 μmol Tris, 1 μmol $MgCl_2$, 0·2 μmol ATP, 0·4 μCi γ-^{32}P-ATP and washed particles derived from 4 mg epidermis (total volume 0·4 ml, pH 7·4).

'Total' (Mg^{++} and Na^{+}-K^{+} activated) ATPase. As above, plus 40 μmol NaCl and 12 μmol KCl.

Duplicate samples and reagent blanks were incubated for 30 min at 37°C. The reaction was stopped with 0·6 ml TCA (10% w/v), and inorganic phosphate extracted by the method of Berenblum & Chain (as described by Lindberg & Ernster, 1954). The activity of the extract was measured by scintillation counting.

Our results are summarized in Table 1; there is no significant difference between any of the groups at a level $P<0·05$. Two conclusions thus emerge: first, that there is little or no stimulation of the

TABLE 1. Mg^{++} stimulated and total ATPase activities of washed particulate preparations of epidermis

Material	No. of specimens	Mg^{++} activated ATPase	Total ATPase
Healthy controls	8	73 ± 21	85 ± 30
Psoriatic, uninvolved epidermis	5	86 ± 29	83 ± 14
Psoriatic, lesion	5	69 ± 19	88 ± 11

All values (mean ± s.d.) are expressed as nmol phosphate released per min per g fresh weight epidermis.

ATPase activity of our preparations by Na^+ and K^+, and second that the membrane-bound ATPase activity of psoriatic epidermis is, *per se*, normal.

Mahrle & Orfanos (1974) comment that 'it remains open to question as to whether the decreased ATP hydrolysis in psoriasis is due to a decreased activity of membrane-bound ATP-hydrolysing enzymes or to a limited access of substrate on the cell surface'. Our present findings seem to provide an unambiguous answer to this question; the possibility of diminished substrate access remains to be explored.

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British Journal of Dermatology (1975) 93, 471.

Inhibition of lysosomal enzymes by dapsone

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Accepted for publication 24 April 1975

Massive doses of vitamin A lead to excessive degradation of tissue chondroitin sulphate, presumably mediated via the release of lysosomal hydrolases. Barranco (1974) has shown that dapsone (diaminodiphenyl sulphone) prevents this response, and has speculated that the drug may act by the direct inhibition of lysosomal enzymes.

We have therefore investigated the effect of dapsone on fourteen lysosomal hydrolases *in vitro*. Extracts of guinea-pig epidermis were prepared as before (Mier & van den Hurk, 1975a). Dapsone (generously donated by A.C.F.Chemiefarma, Maarssen, The Netherlands) was dissolved in dimethylsulphoxide (DMSO) at 40 mg/ml, and diluted with water to 400 μg/ml. Further dilutions of 40 μg/ml and 4 μg/ml were made using 1% aqueous DMSO. Assay mixtures consisted of 0·1 ml buffered substrate, 0·05 ml epidermal extract or BSA (reagent blank) and 0·05 ml dapsone solution or 1% DMSO (control); the individual substrates, reaction conditions and assay techniques have been described previously (Mier & van den Hurk, 1975a–c). All measurements were carried out in duplicate. After subtraction of the appropriate reagent blank, inhibition was calculated as a percentage of the activity of the control.

No significant inhibition of any enzyme was observed at final dapsone levels of 1 or 10 μg/ml. At 100 μg/ml, six glycosidases and arylamidase were significantly inhibited (Table 1). Acid phosphatase (EC 3.1.3.2.), pyrophosphatase (EC 3.1.4.1), arylsulphatases A and B (EC 3.1.6.1), β-galactosidase (EC 3.2.1.23), cathepsin B, (EC 3.4.4.-) and cathepsin C (EC 3.4.4.9) were unaffected.

These findings would, at first sight, appear to support the hypothesis of Barranco (1974). However, difficulties arise when comparing our data with the situation *in vivo*. In particular, the blood level following a normal therapeutic dose of dapsone is only of the order of 5 μg/ml (Chang, Wolcott &

TABLE 1. Percentage inhibition of individual hydrolases at a final dapsone concentration of 100 μg/ml

EC number	Enzyme	% inhibition
3.2.1.20	α-glucosidase	25
3.2.1.21	β-glucosidase	38
3.2.1.22	α-galactosidase	10
3.2.1.24	α-mannosidase	39
3.2.1.30	β-acetylglucosaminidase	7
3.2.1.31	β-glucuronidase	24
3.5.1.–	Arylamidase	19

Doull, 1954); we must therefore postulate at least a 20-fold concentration if Barranco's thesis is to remain valid. Specific uptake of certain dyes, drugs and other materials by lysosomes is, of course, well documented. Whether dapsone undergoes a similar fate remains to be investigated; unless this can be demonstrated, an alternative mechanism must be sought for the therapeutic effects of this compound.

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INDUCED TOLERANCE IN PHYSICAL URTICARIAS

SIR, The interesting account of heat urticaria treated by inducing tolerance to heat (Leigh & Ramsay, 1975) stimulates me to report the following:

A 62-year-old heating engineer developed a rash at the age of 42 which has persisted ever since. When seen in 1975 he was found to have marked dermographism. He stated that a number of his paternal relations suffered from the same disability. His father, an Irish farmer, had told him 'a brush was as good for a horse as a feed of oats'. The patient adopted this advice for himself and brushed his trunk and limbs before going to bed each night using a hair brush, as he put it 'a brush for him was as good as a pill'. This seems to be an example of factitial urticaria treated by induced tolerance to mechanical trauma.

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CUTANEOUS PROTOTHECOSIS

SIR, We should like to report on the progress of a case of human cutaneous protothecosis previously recorded in the *British Journal of Dermatology* (Mars *et al.*, 1971).

The patient, a black African labourer, presented with cutaneous protothecosis involving the forehead and scalp. The lesions had been growing slowly over the course of a year, and *Prototheca wickerhamii* was isolated from them. Initially following treatment with potassium iodide (1 g per day for 10 weeks) an apparent response with regression of the lesions was noted, although the organisms continued to be cultured.

Following discharge from hospital the patient became lost to follow-up and presented a year later with recrudescence and extension of the original lesions. He was in good general health and was unwilling to be admitted to hospital, which precluded the use of the more toxic anti-protothecal agents such as pentamidine isethionate and amphotericin B. A further course of potassium iodide (1 g per day for 3 months) was without effect and as the patient still refused in-patient treatment he was given a vitamin B preparation (Adamol tablets, 6 tablets daily) as a placebo and urged to continue attendance as an out-patient.

Six months later the lesions had decreased remarkably in size and a year later had cleared completely. Another year later (3½ years after presentation) there had been no further recurrence. At no time was there clinical evidence of vitamin deficiency.

Therapy in protothecosis has often proved difficult. The first two reported cases (Davies & Wilkinson, 1967; Klintworth *et al.*, 1968) both proved recalcitrant to a variety of treatments, whereas subsequent more localized cases (unconfirmed by culture) responded to surgical excision (Tindall & Fetter, 1971; Nosanchick & Greenberg, 1973). A recent case of systemic protothecosis (Cox *et al.*, 1974) showed a deficiency of cell-mediated immunity and responded to treatment with amphotericin B and transfer factor. Our patient showed no evidence of defective cell mediated immunity: lymphocyte transformation with phytohaemagglutinin was normal; positive delayed hypersensitivity skin tests to tuberculin and to a prototheca antigen were elicited. At no time was there clinical evidence of vitamin B deficiency.

We feel that in our patient the regression of this rare and slowly progressive disease can be regarded as spontaneous and may be worthy of record as the first such instance.

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Book Reviews

Atopic Dermatitis. G. Rajka (1975) Vol. 3. in 'Major Problems in Dermatology'. London: W.B. Saunders. Pp. 165, Illustrated. Price £5.50.

The basic pathogenesis of atopic dermatitis is probably more obscure now than it has seemed for a long time. Opinions on the role of the immediate weal type of allergy have oscillated or vacillated for the past 50 years; and now so much more is also known about disturbances in delayed type hypersensitivity and about the other numerous non-allergic aspects of the disease. Professor Rajka's monograph (in the series 'Major Problems in Dermatology') appears at a very good time to bring together a vast amount of material about the nature of the disease—but not perhaps at a time when the reader can feel that he has all the answers therapeutically. The author has an unrivalled experience in clinical and fundamental investigation of atopic dermatitis, notably his work on the role of allergy and on the cardinal importance of itching. (His well documented account of itching in the mouth will surprise many.) He stresses that this is a monograph on atopic dermatitis which makes it completely different from a monograph on atopy. It is probably the fullest available account, and discusses all aspects of this multi-factorial disease, except for the work on atopy in animals. Obvious openings for research are apparent on many pages. The author is often prepared to hazard an opinion on controversial issues and even when he is not, one can often only agree with his silence. The overall result is a work which must appeal to clinicians and laboratory workers alike.

R.H.Champion

Dermatology—Diagnosis and treatment of cutaneous disorders W.D.STEWART, JULIUS L.DANTO and STUART MADDIN (1974) Second edition. Saint Louis: The C.V.Mosby Company. Pp 537. Price £10.70.

With rapid changes in many fields of dermatology and several excellent and up-to-date textbooks and synopses of dermatology directed mainly to students and general practitioners available, one expects major changes in layout and content if a new edition of a book is worth producing. The authors of this textbook obviously had this in mind in their new edition when they changed the title of the previous edition which was *Synopsis of Dermatology*. The book has been expanded and nearly every chapter contains a section 'pertinent information' which usually gives recent, relevant research and new concepts in a simple, precise form. A useful innovation is the inclusion of references to standard papers at the end of each chapter which remedies an obvious defect in the previous edition. The book is well written and a student could read through it with ease. There are one or two photographs which fall below standard and contribute little, but with these exceptions the illustrations are well chosen and are of excellent quality. The colour plates are all first class and are about the best I have seen in a textbook.

My main criticisms would be, (a) the price could be considered high for the market it is aimed at but is very reasonable in view of the size of the book and standard of the colour plates. (b) There are quite a number of small defects, for instance it is surely essential to say more about the medical treatment of toxic epidermal necrolysis than that 'good treatment is necessary' or to define what is meant by 'adequate surgical excision' for melanoma. In the contact dermatitis section cement is not mentioned under 'most common source' of sensitization under chrome, and would it normally be common practice to patch test with metals 'as is' which is recommended in the section on patch testing? There are quite a large number of similar small criticisms of this type which one might make. (c) A few chapters fall below the general standard, mainly those on histiocytosis which was unchanged from the previous edition, where no clear difference is made between the different types and their different treatment, and porphyria, where the different types are well described but the differences in treatment are not clear.

However, I found it an improved, lively and readable book and would be happy to recommend it to medical students and general practitioners who wanted to improve their dermatology.

D.BURROWS

Haut-veränderungen by inneren Krankheiten. I.PASTINSZKY and I.RACZ (1974) second edition. Berlin: VEB Verlag Volk und Gesundheit. Pp. 1492, illustrated.

The first edition of this book was published in 1965. The second edition is expanded to two volumes (also available in Hungarian). It is the outcome of hard work (no energy crisis here) by the two distinguished authors who are from Debrecen and Budapest respectively. The first edition was soon taken up and the second will become justly popular especially in the German-speaking dermatological world. It aims 'to present a correlation between cutaneous appearances and physiological and pathological processes in the organism' and is based on over 10,000 listed references selected without national bias from the world literature up to 1973.

The faults of the book may be in the very achievement of the authors in packing so much information into its pages. Perhaps they have not been sufficiently selective and, at times, one cannot see either the wood or the trees from lack of use of the chopper. Thus quite a few reported associations between skin and other conditions which they describe may be fortuitious. The illustrations are in black and white and have been contributed by many colleagues from other centres. A few are not clear. Inevitably, rarities are discussed and this means plenty of ponderous, eponymous and Latin labels.

Presentation, printing, format and weight of the two volumes are excellent. The index is complete and accurate. Altogether, the authors and publishers have done a very good job. The work, however, is not for the bedside but should serve well for reference on the library shelves.

M.FEIWEL

British Journal of Dermatology (1975) **93**, 477

Review

Dispersal of skin microorganisms*

Studies on the dispersal of skin microorganisms can be considered under three headings: investigations of the skin surface and of the mechanism of desquamation; hospital or industry based studies relating to the importance of dispersal; studies aimed at understanding and controlling dispersal.

Skin as a source of organisms

The average human has a skin area of about 1·75 m^2 divided approximately as follows: legs 18% each, trunk 37%, arms 9% each and head 9%; specialized areas such as the perineum and axillae account for less than 2% of the total skin area. The skin scales which compose the surface are about $30 \times 30 \times 3$–5 μm and consequently some 10^8 are needed to complete the skin surface. Females have larger scales on average than do males (Plewig, 1970). A complete layer of cells is lost and replaced on average about every 4 days (Halprin, 1972; Jansen, Hojyo-Tomoko & Kligman, 1974), hence we all disseminate more than 10^7 particles of skin every day. Although clearly bathing or showering will remove very many scales by mechanical means, Sciple, Riemensnider & Schleyer (1967) found that natural walking movements released about 10^4 scales per minute. Scales are also released even when standing naked, presumably because the flakes tend to curl up and become detached when dried on one side only. They are then carried up in the airstream so elegantly demonstrated by Lewis *et al.* (1969). Unpublished studies have shown that about 10 mg of skin is deposited in the clothing in 2 hours.

Microbial skin colonizers are more numerous in some areas than others, for example the head and thorax carry more microbes than the limbs; males are more heavily colonized with aerobes than are females, though anaerobes appear to be more equally distributed between the sexes (Table 1). A further complicating factor, however, is that the microorganisms are not spread out equally over their habitat but live in discrete microcolonies which may be small, of the order of 10^2 cells, or large, of the order of 10^5 viable cells. These colonies may be relatively far apart on the skin and this accounts for the fact that only about 10% of squames carry viable microorganisms (Noble & Davies, 1965).

In disease, especially eczema, psoriasis and pityriasis rosea, the skin may be densely colonized by *Staphylococcus aureus* and/or *Streptococcus pyogenes*. These organisms are then dispersed on skin scales and such persons may contaminate their environment with these pathogens (Noble & Davies, 1965; Selwyn & Chalmers, 1965; Noble, 1971). Any organism found on the skin surface will be dispersed including fluorescent corynebacteria (Somerville-Millar, personal communication), fungi (Clayton & Noble, 1963; Noble, Lidwell & Kingston, 1963) and presumably viruses. There is some evidence that smaller skin particles may be involved in dispersal by dermatology patients despite the large fragments of desquamated skin which are a feature of some skin diseases, though it is not clear whether this is a reflection of the disease process or of the greater degree of colonization of the skin. Some individuals may be a potent source of organisms, such as the psoriatic anaesthetist recorded by Payne (1967) and the eczematous porter (Ayliffe & Collins, 1967). It must be remembered, however,

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* Paper based on that presented at the Nordic Association for Contamination Control, Gothenburg, 2–4 April 1975.

TABLE 1. Geometric mean count per square centimetre of skin surface in eleven normal healthy males and eleven normal healthy females

Site	Aerobic flora		Anaerobic flora	
	Males	Females	Males	Famales
Forehead	2075	1225	8000	13500
Sternum	2125	165	50000	3500
Subclavicular area	350	130	18500	2275
Centre back	450	155	67500	7500
Shoulder	128	48	1025	1075
Deltoid area	118	65	57	127
Forearm	250	35	9	13
Palm	98	155	33	85
Lower axilla	500	92	14	12
Lumbar area	300	33	178	142
Periumbilical area	850	175	55	80
Thigh upper front	325	140	9	35
Thigh lower front	350	67	14	16
Thigh back	325	82	4	5
Shin	190	77	7	8
Calf	173	20	2	5
Dorsum of foot	80	122	3	10
Sole	22750	675	10	4

Based on data summarized in Somerville & Murphy (1973).

that whilst patients with skin disease are frequently important sources of pathogenic microorganisms, many dispersers have been discovered who have no clinically apparent epidermal abnormality.

The hair acts as a mechanical sampler of the environment and several studies have recorded the propensity of the hair to carry *Staph. aureus* and other pathogens (Summers, Lynch & Black, 1965; Black, 1966; Noble, 1966; Black, Bannerman & Black, 1974). Opinion is divided as to the role of the hair in dispersal.

Definition of a 'disperser'

In a study of dispersal in hospital wards Noble (1962) defined as a disperser of *Staph. aureus* a patient who contributed more than 0·2 *Staph. aureus* particles per cubic foot (6/m^3) to the air of a ward housing many patients. Since the mean air-count of all organisms was of the order of 27 viable particles per cubic foot (800/m^3), this figure was equivalent to about 1% of the total flora. Noble & Davies (1965) later adopted a less stringent definition requiring a 'disperser of *Staph. aureus*' to disseminate this organism as more than 1% of his total flora whilst undressing in a cubicle of about 100 cubic foot (2·8 m^3) capacity. Bethune *et al.* (1965) defined as a disperser of *Staph. aureus* one who disseminated more than 10 *Staph. aureus* particles into the air of a 100 cubic foot chamber during exercise; Blowers, Hill & Howell (1973) later amended this to 4 *Staph. aureus* particles/m^3. This is clearly of the same order as the definition proposed by Noble & Davies.

Dispersal is, of course, a very variable event. Even in studies of the normal flora, where presence or absence of a specific organism does not influence the result, the standard deviation of the mean air count during undressing is of the order of 50% (Table 2).

TABLE 2. Dispersal of organisms by various individuals. Throughout each investigations each individual used the same hygienic regimen

Individual	Sex	No. of samples	Mean air count during undressing	S.D.	S.D. as % of mean
1	F	9	825	216	26
2	F	6	669	240	36
3	F	7	998	375	38
4	F	6	886	340	38
5	M	10	657	270	41
6	F	10	2315	1050	45
7	M	20	1320	645	49
8	F	9	847	450	53
9	F	9	1720	955	56
10	M	6	1316	830	63
11	M	7	582	535	92

Based on material summarized in Wilson (1970).

Dispersal as a hazard

Early studies of dispersal include those of deForest & Kerr (1945) who reported a case of eczema as a source of streptococci; Loosli and his colleagues (1950) who identified 'skin dispersers' as sources of streptococci in wards for neonates; and those of Hare and his co-workers who firmly implicated patients with eczema and mycosis fungoides as potent sources of airborne microorganisms (Hare & Ridley, 1958; Hare & Cooke, 1961). Duguid & Wallace (1948) had already identified dust from clothing as a means by which organisms became airborne and distributed about the environment.

Studies of staphylococcal cross-infection in hospital wards gave a boost to studies of dispersal and acquisition. *Staph. aureus* was shown to be spread round hospital wards, appearing in the noses and less frequently in the lesions of surgical patients (Williams *et al.*, 1962; Noble, 1962). Debate on the nature of the 'fibre nuclei' believed to be responsible for dissemination from bedclothes led to the rediscovery of skin scales as the raft on which microorganisms became airborne (Davies & Noble, 1962). A raft of some nature was predictable from the aerodynamic behaviour of the particles. Although on average only 4 viable *Staph. aureus* cells appeared to make up each airborne particle (Lidwell, Noble & Dolphin, 1959) the particles were shown to be about 14 μm in equivalent diameter; a particle big enough to be composed of some hundreds of cocci (Noble, Lidwell & Kingston, 1963). It should perhaps be emphasized that, for reasons of mathematical convenience, airborne particles are best considered as spheres having unit density. Thus on average the $30 \times 30 \times 3$–5 μm flakes behave in the same fashion as spheres of 14 μm diameter.

Although the mean is around 14 μm equivalent diameter there is a considerable range in size of the individual particles. Table 3 shows the range in sizes derived from data summarized in Noble & Davies (1965). The range is broadly comparable with that reported by Clayton & Noble (1963) for particles bearing fungi obtained during examination of patients in a mycology clinic. The median diameters are greater than those obtained for skin scales alone (8 μm, Davies & Noble, 1962); this is presumably a reflection of the greater chance that larger particles will bear bacteria. May & Pomeroy (1973) published size distribution data which showed that 45% of particles from males and 28% of particles from females were too large to remain airborne (over 60 μm equivalent diameter). When air-

TABLE 3. Size distribution of airborne particles in dispersal experiments based on data summarized in Noble & Davies (1965)

Population	Normal flora				*Staph. aureus*	
	Normal adult males	Normal adult females	Adult surgical patients	Infant eczema patients	Adult surgical patients	Infant eczema patients
No. of individuals	12	12	21	8	3	8
Total colony forming units contributing	76614	16746	263774	28940	10048	18213
Cumulative % of particles greater						
than 18·2 μm	43	39	28	30	34	11
9·6 μm	79	75	55	36	72	57
4·2 μm	95	94	89	95	90	86
Median equivalent diameter (μm)	17	16	13	14	14·5	11

borne particles only were considered, about 27% were over 20 μm and 11% less than 5 μm, values equivalent to those shown in Table 3.

That these airborne particles were in fact skin scales was necessarily an inference for there is no specific stain for keratin; yet the particles were composed of protein and contained some fat, they bore human blood group substances (demonstrated by the Coombs' technique (Coombs, Bedford & Rouillard, 1956)), they carried human skin organisms and finally they *looked* like skin scales (Davies & Noble, 1962). Little else could be imagined which would fit these criteria. The way was then open for studies on methods of preventing dispersal. The principal lines of attack were the use of antibacterial agents and of squame-proof clothing.

Dispersal from hair. Dineen & Drusin (1973) reported that dispersal of *Staph. aureus* from the scalp of two individuals in an operating theatre resulted in post-operative wound infection. One of the two individuals had frank scalp lesions which drained pus prior to the epidemic of infection. A more subtle hazard is presented by the fashion for wearing beards and has stimulated much editorial comment but little investigation. Barbeito, Mathews & Taylor (1967) found that nuzzling into an infected beard could transmit infection to guinea-pigs. However, Dr A.G.M.Huysmans-Evers (personal communication) has found that thirteen individuals of a total of eighty-four carried *Staph. aureus* on the beard and that seven of these thirteen dispersed this organism and were thus a potential hazard.

Reduction of dispersal. Antibacterial agents such as hexachlorophane and trichlorocarbanilide (TCC) when used regularly in a soap formulation have a statistically significant effect in reducing the skin flora (Solberg, 1965; Speers *et al.*, 1966; Wilson, 1970). There has, however, been a move away from the use of these compounds following the reports of brain damage resulting from absorption of hexachlorophane. Antibiotic treatment of carrier sites also reduces the dispersal of pathogens (Varga & White, 1961; Solberg, 1965). Redesign of clothing has been pursued with more vigour. Normal fabrics have very large pores between the woven threads and these pores permit the easy passage of skin scales. 'Ventile' fabric, however, has a very close weave which does not permit the passage of scales and so reduces dispersal. Redesign of operating theatre clothing itself, with less loose openings, also reduces the bacterial load disseminated during operations (Bernard *et al.*, 1965; Bethune *et al.*, 1965; Charnley & Eftekhar, 1969).

It is clear from the work of May & Pomeroy (1973), Hill, Howell & Blowers (1974) and Mitchell & Gamble (1974) that clothing that specifically occludes the perineum significantly reduces dispersal. Yet, as Ayliffe, Babb & Collins (1974) pointed out, this is of value only when the perineum is the prime source of organisms. Where nasal carriers are concerned (White, 1961; Solberg, 1965) occlusive clothing is of little value and Ayliffe and his colleagues suggested that it be reserved for high risk operations, such as hip replacement (Charnley, 1973).

The hazard in dispersal of *Staph. aureus* or any other potential pathogen lies in the chance that the organism will find its way into an otherwise sterile field. In industry Sykes (1970) has pointed out that a room can be designed and maintained 'virtually sterile' until it is occupied by humans; such conditions are required for sterile filling of injectable materials. Favero *et al.* (1966) have reported essentially similar studies whilst Behagel & Berg (1973) reported on the difficulty of maintaining staphylococcal cultures free from the risk of contamination by phage derived from strains carried by personnel. In a hospital situation the gravest risk is in the operating theatre (Payne, 1967; Ayliffe & Collins, 1967; Charnley, 1973), but there is also a general risk in the excessive dispersal of organisms into the air of wards (Hamburger, Green & Hamburger, 1945; Noble, 1962; Schaffner *et al.*, 1969). In operating theatre outbreaks it is fairly easy to document and ascribe infection to one individual; in the instance quoted by Payne (1967) there were four deaths amongst the thirty-three patients involved and the source was an anaesthetist with psoriasis. It is less easy to be specific in ward outbreaks though Lidwell & Brock (1973) have demonstrated the relationship between dispersal and nasal acquisition.

Intrinsic interest in dispersal

Although in total the process of dispersal is clearly a mechanical one, there have been a number of curious facets which created an interest in dispersal as a research topic.

In the studies carried out by Shooter and Blowers and their colleagues it was found that, following showerbathing, there was often an *increase* in the dispersal of non-pathogenic organisms, despite the fact that showering clearly removed thousands of organisms from the skin. This may best be explained by reference to the microcolonies of bacteria on the skin surface. During washing, many of the bacteria in the colonies are removed mechanically but others are spread out over the skin surface thus contaminating more scales than previously. Sebum is also removed during washing which may leave the skin temporarily more susceptible to desiccation so that scales may dry, curl up and become free more readily than when sebum is abundant. After a short period—as little as 2 hours—the skin returns to normal. This is illustrated in the work of Holt (1971) who found that the average microcolony size decreased following washing or bathing, as did the count of total bacteria, but that temporarily the contact count, which measures the number of independent viable units, increased. This increase in dispersal of normal flora following showering led to suggestions that surgeons abandon the traditional shower before operation, despite the demonstration by Cleton, van der Mark & van Toorn (1968) that contaminant pathogenic organisms, which would include those acquired during ward rounds, etc. are all removed by the shower.

Another curious observation was that normal healthy males dispersed more than did females, especially when clad in 'street' clothing. The sex difference was reduced when the same clothing or no clothing was worn but it persisted (Table 4). Although Noble & Davies (1965) could not ascribe differences in dispersal to differences in size between males and females, a re-examination of their

TABLE 4. Sex ratio of total flora dispersal under different degree of clothing. All air samples in a cubicle

Method of measuring dispersal	Clothing	No. of samples	No. of individuals	Ratio male/female	Authors
Undressing	Street	42	42	4·1	Noble & Davies (1965)
	Cotton pyjamas	42	42	1·4	
Walking on spot	Street	46	10	3·3	Speers *et al.* (1965)
	Nil before shower	20	10	2·0	
	Nil after shower	46	10	1·8	
Exercise	Street	10	10	4·7	Doig (1972)
	Swim suit	10	10	1·0	
Exercise, rub skin or clothing	Street	28	28	1·3	May & Pomeroy (1973)
	Nil	28	28	5·2	
Exercise	Street	16	9	5·2	Ballard & Lidwell (personal communication)
	Nil	16	9	4·9	
Undressing	Street	54	9	1·22	Wilson (personal communication)
Undressing	Street	72	72	3·6	Noble, *et al.* (in preparation)

data in the light of Plewig's (1970) report that females have larger scales than males is interesting. It can be calculated that, on the basis of differences in squame size, males ought to disperse about 1·25 times as many squames as do females. The skin areas of the people studied by Noble & Davies were available and, since the males were on average larger, a further factor of 1·16 might be added. Males ought then to disperse 1·45 times as many scales as do females; the sex ratio for dispersal of scales found by Noble & Davies was 1·34. There is, however, a further factor to be taken into account when we discuss dispersal of bacteria. It is that males are more heavily colonized with bacteria than are females (Table 1). In a study of surgical personnel (Noble *et al.*, in preparation) it was found that, although males dispersed about 3·5 times as many bacteria as do females, the sex difference disappeared if allowance was made for the denser colonization of males.

Perhaps more striking is the observation that very few healthy young women disperse *Staph. aureus*. The most extensive studies have been those of Hill, Howell & Blowers (1974) who have detected forty-five dispersers of *Staph. aureus* amongst 389 men but only eight amongst 613 women. It seemed possible that this was under hormonal influence but examination of 100 post-menopausal women has also failed to reveal more than five dispersers (Mitchell & Gamble, 1974).

Others, however (Solberg, 1965; Oud, 1969; Solberg, Bruun & Bøe, 1972), have found female dispersers of *Staph. aureus* though they are much less active than males. On a rank order basis males significantly outrank females. This male predominance does not extend to patients, however; studies on airborne staphylococci have failed to reveal differences between males and females in the degree of dissemination in hospital wards (Lidwell & Brock, 1973). Some studies help to throw light on this phenomenon. The classic work of Solberg (1965) showed that the degree of dispersal of *Staph. aureus* was directly related to the degree of contamination of the skin with this organism. Blowers' investigations had previously shown that, in experiments where persons in operating theatre clothing exercised in a cubicle, dispersal was mainly from below the waist. Recent studies in the Netherlands (Noble *et al.*, in preparation) showed that, in experiments in which undressing was the means of dispersal, the correlation (analysed by a forward stepwise regression analysis) between dispersal and body site was most significant for the abdomen and thighs in males and for the shin in the females (perhaps due to the 'cheese-grater' effect of tights and stockings described by Mitchell & Gamble, 1974). If this difference is found to be true for studies of exercising rather than undressing, the predominance of males in dispersing *Staph. aureus* might be ascribed simply to greater contamination of the thighs than the shins with perineal organisms.

There is still much that remains to be discovered in relation to dispersal. Why are males more heavily colonized with skin bacteria than are females? Why do some individuals without skin disease carry *Staph. aureus* on the skin for short—or long—periods? Can differences in clothing account for differences in dispersal pattern, and finally can dispersal of potential pathogens be prevented without recourse to hot, perhaps uncomfortable, operating theatre clothing and without the application of apparently harmful skin degerming compounds?

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News and Notices

University of Munich

The VIIIth Postgraduate Course in Practical Dermatology and Venereology, organized by the Department of Dermatology, University of Munich in co-operation with the ‘Verband der Niedergelassenen Dermatologen Deutschlands e.V.’, will be held in Munich from 26 to 30 July 1976. The official language is German. There will be no simultaneous translations.

The main themes include: (1) Dermatoses in childhood: (2) Treatment nodalities for basal cell carcinoma, squamous cell carinoma and keratoacanthoma; (3) Diagnosis of allergies; (4) Proctology; (5) Immune-dermatoses; (6) ‘From symptoms to diagnosis’; (7) Acne; (8) Treatment; (9) Venereal disease.

Chairman: Professor O. Braun-Falco. Information requests to Professor S. Marghescu, Dermatologische Universitätsklinik München, D-8000 München 2, Frauenlobstrasse 9, West Germany.

International Society of Paediatric Dermatology

The International Society of Paediatric Dermatology has been formed recently by a group of Dermatologists and Paediatricians. Those interested to become members of the Society can be either Paediatricians or Dermatologists, or both. Officers of the Society are: Dr Ramon Ruiz-Maldonado, President; Dr Lawrence Solomon, Secretary; and Dr Coleman Jacobson, Treasurer.

For further information write to: L. Solomon, M.D., P.O. Box 6998, Chicago, Ill.60680. U.S.A.

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In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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British Journal of Dermatology, Volume 93, Number 4, October 1975

CONTENTS

Clinical and Laboratory Investigations

PAGE

Pigmented Basal Cell Epithelioma: Light and Electron Microscopic Studies on Tumours and Cell Cultures 361
STANLEY S. BLEEHEN

Pigmentary Demarcation Lines: Comparison of Negroes with Japanese 371
VICTOR J. SELMANOWITZ AND JAMES M. KRIVO

Absorption and Biotransformation of Topically Applied 8-(methylthio)cyclic AMP: Studies in Dogs and Humans 379
JACQUES DREYFUSS, TERRENCE W. MISCHLER, EDWARD S. WEISS, JAMES M. SHAW AND ERIC C. SCHREIBER

Lysosomal Hydrolases of the Epidermis. 2. Ester Hydrolases 391
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK

Median Rhomboid Glossitis: Candidiasis and not a Developmental Anomaly 399
BRIAN E. D. COOKE

Plane Xanthomata Associated with Multiple Myeloma and Hyperlipoproteinaemia 407
K. J. MARIEN AND G. SMEENK

Dermo–epidermal Adhesion and its Effect on Epidermal Structure in the Mouse 417
WILLIAM S. BULLOUGH AND JOHANNA U. R. DEOL

The Differential Binding of Antibody from the Sera of Patients with Pemphigus and Pemphigoid to Isolated Guinea-pig Epidermal Cells 425
W. D. BRIGDEN AND H. E. AMOS

Immunohistological Studies, with Anti-connective Tissue and Anti-immunoglobulin Antisera, of the Skin in Lupus Erythematosus and Scleroderma 431
N. R. ROWELL AND D. G. SCOTT

The Deoxyribonucleic Acid (DNA) Skin Test in Systemic Lupus Erythematosus. 1. Clinical Evaluation 443
E. A. JOHANSSON, U. KIISTALA, K-M. NIEMI, H. HALME AND A. LASSUS

The Deoxyribonucleic Acid (DNA) Skin Test in Systemic Lupus Erythematosus. 2. Histological Findings 451
E. A. JOHANSSON, K-M. NIEMI AND A. LASSUS

An Improved Method of Assessing Topical Corticosteroid Activity 459
H. BARNES, P. M. GAYLARDE, A. P. BROCK AND I. SARKANY

Case Report

Lichen Planus and Ulcerative Colitis 465
EDWARD H. WYATT

Brief Communications

Membrane-bound ATP-hydrolytic Activity of Psoriatic Epidermis 469
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK

Inhibition of Lysosomal Enzymes by Dapsone 471
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK

Correspondence

Induced Tolerance in Physical Urticarias 473
J. S. PEGUM

Cutaneous Protothecosis 473
M. DOGLIOTTI, P. W. MARS, A. R. RABSON AND J. J. RIPPEY

Book Reviews 474

Review

Dispersal of Skin Microorganisms 477
W. C. NOBLE

News and Notices 486

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1. Brit. J. Derm. **92**, 585, 1975
2. Archs. Derm. **86**, 608, 1962
3. Brit. J. Derm. **90**, 197, 1974

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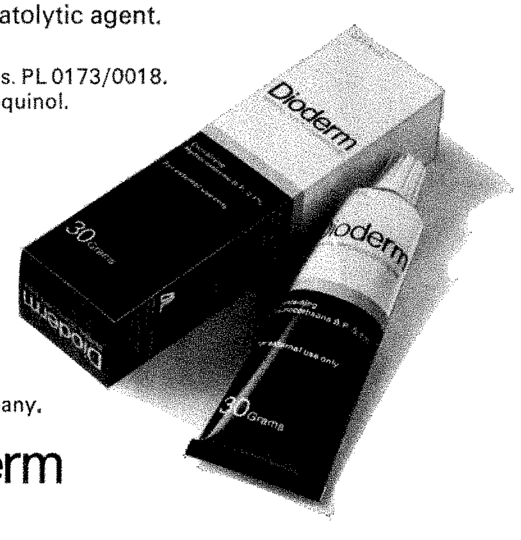

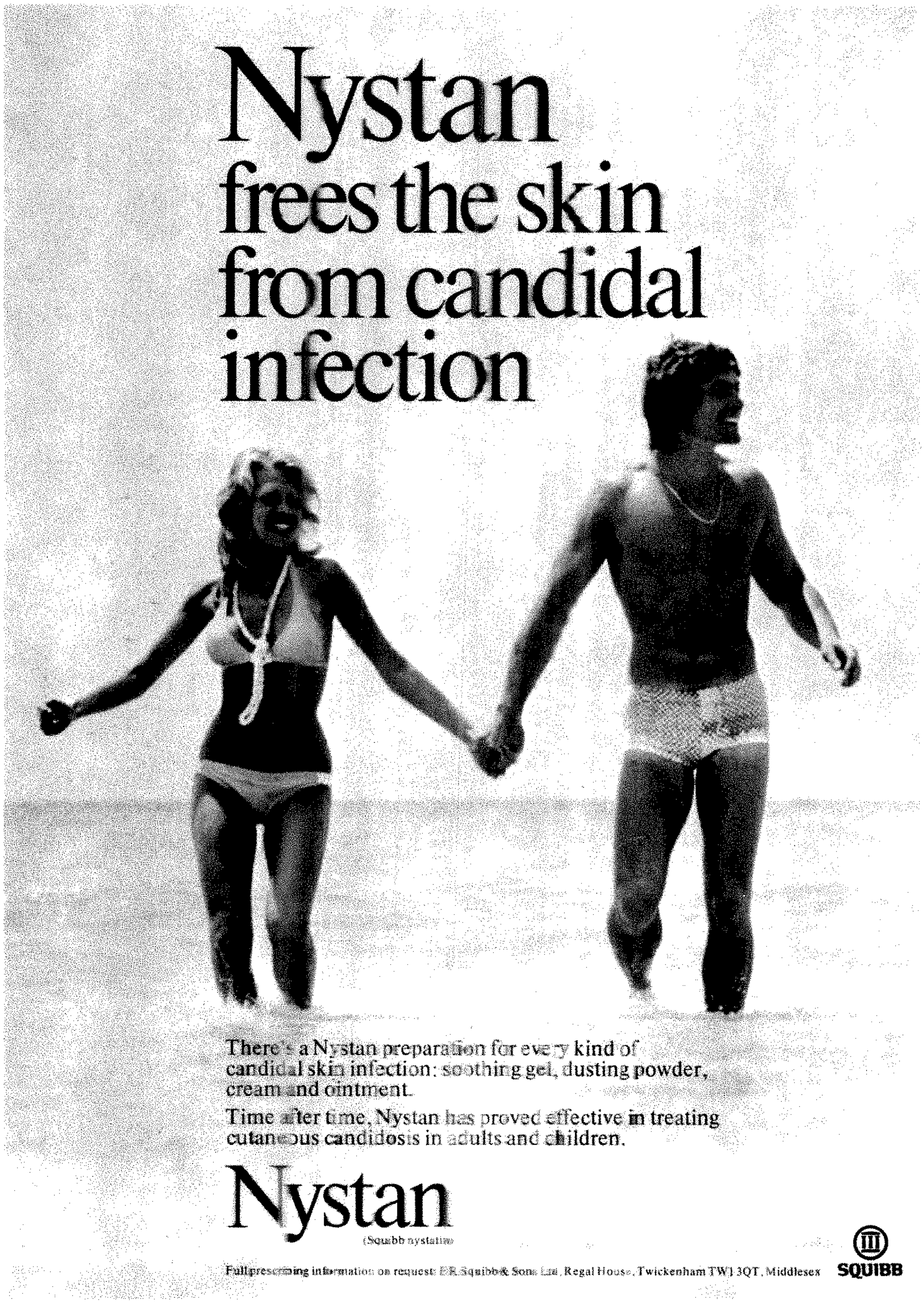

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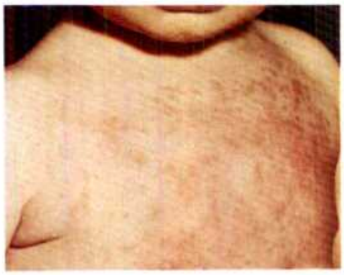

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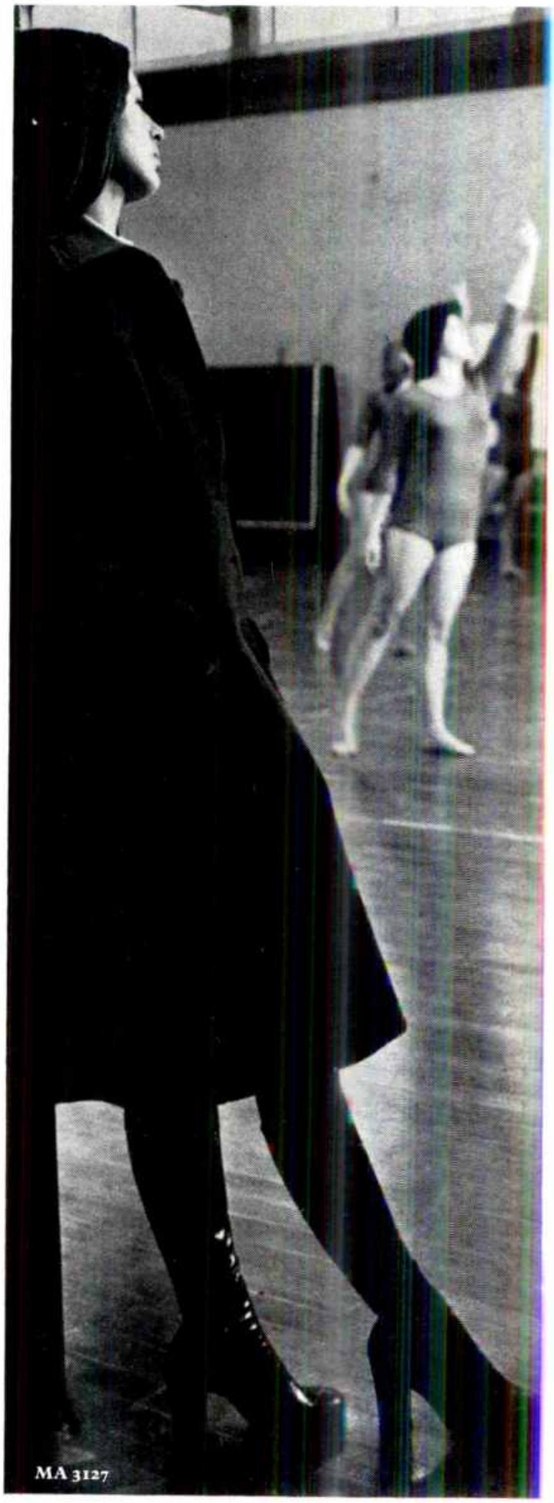

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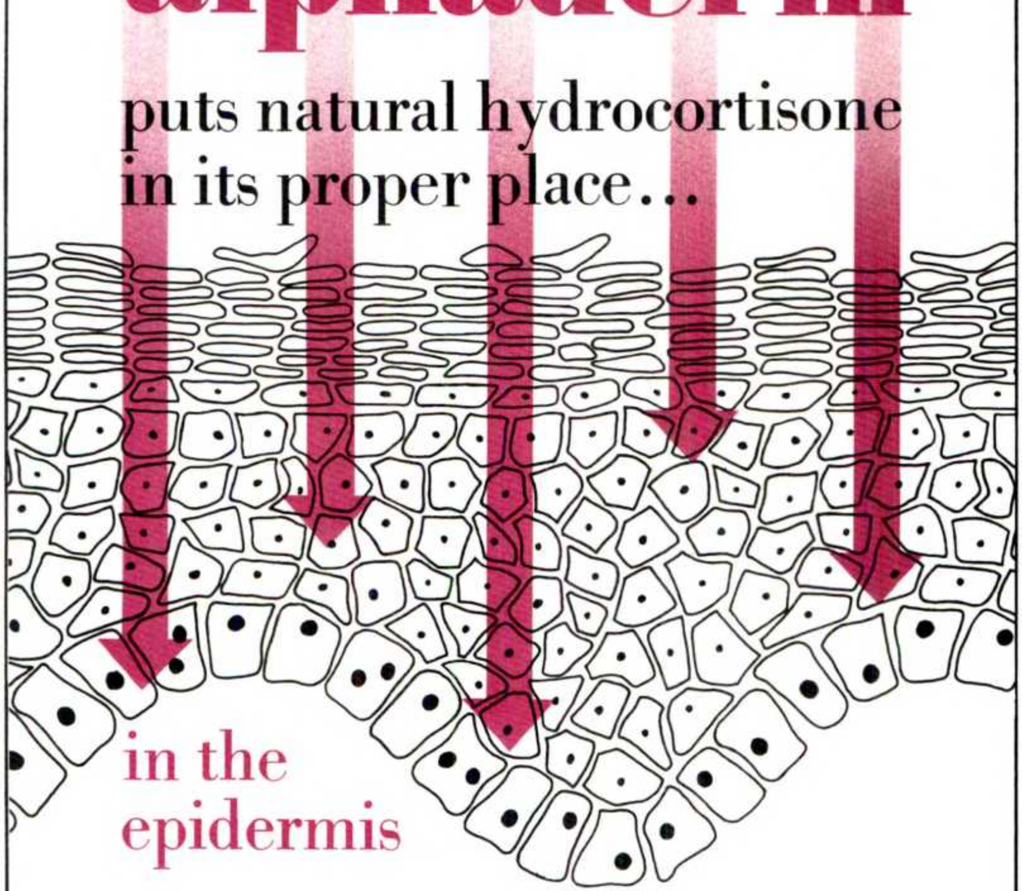

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References
1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91,** 579
2. Jacoby, R.H. and Gilkes, J.J.H. (1974) *Curr.med.Res.Opin.* **2,** 474

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References:
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2. Wagner D.E., et al., Oncology 26 310-316 (1971)
3. Carter S.K., and Friedman M.A., Europ. J. Cancer 8 85-92 (1972)

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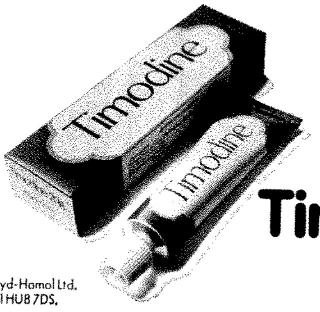

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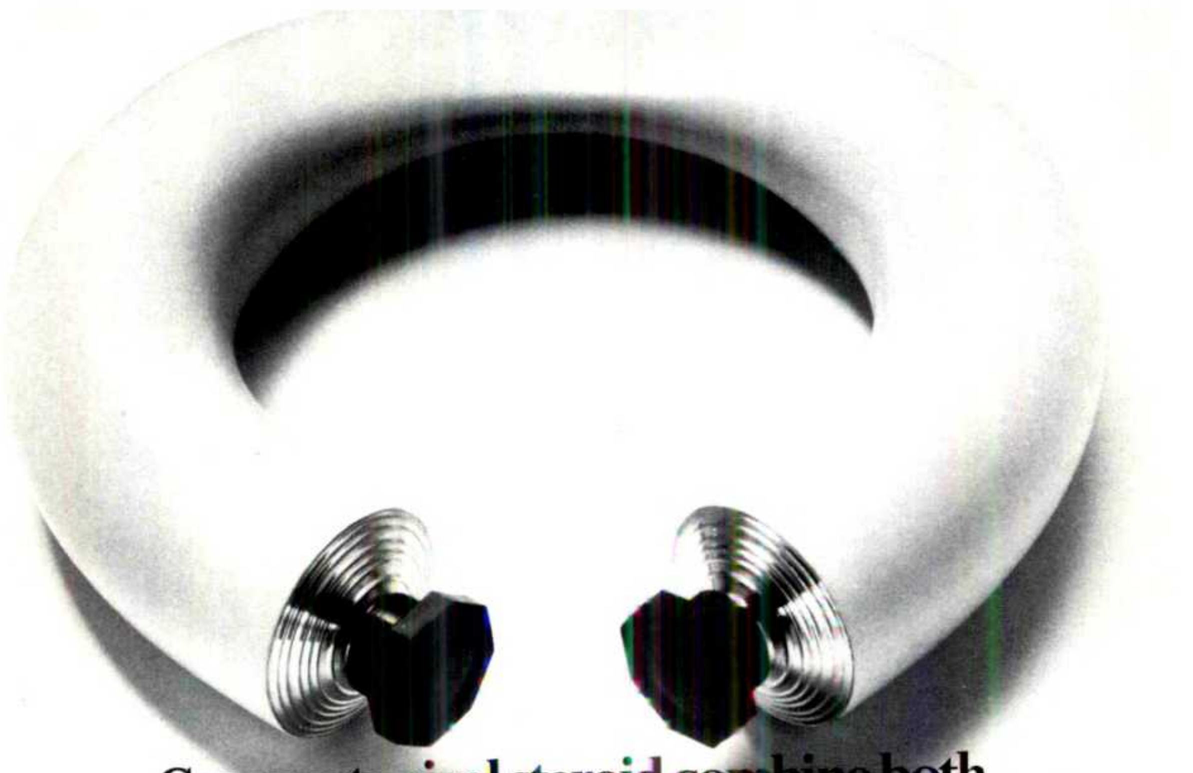

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1 Alexander S. and Lyne C. Brit. J. Clin. Pract. (1973) 27177 2 Ashurst P. J. Brit. J. Clin. Pract. (1972) 23263 3 Polano M. K. et al Brit. J. Derm. (1970) 8393 4 Polano M. K. and Kanaar P. Brit. J. Derm. (1973) 8883 5 Sneddon I. Brit. J. Derm. (1973) 89505

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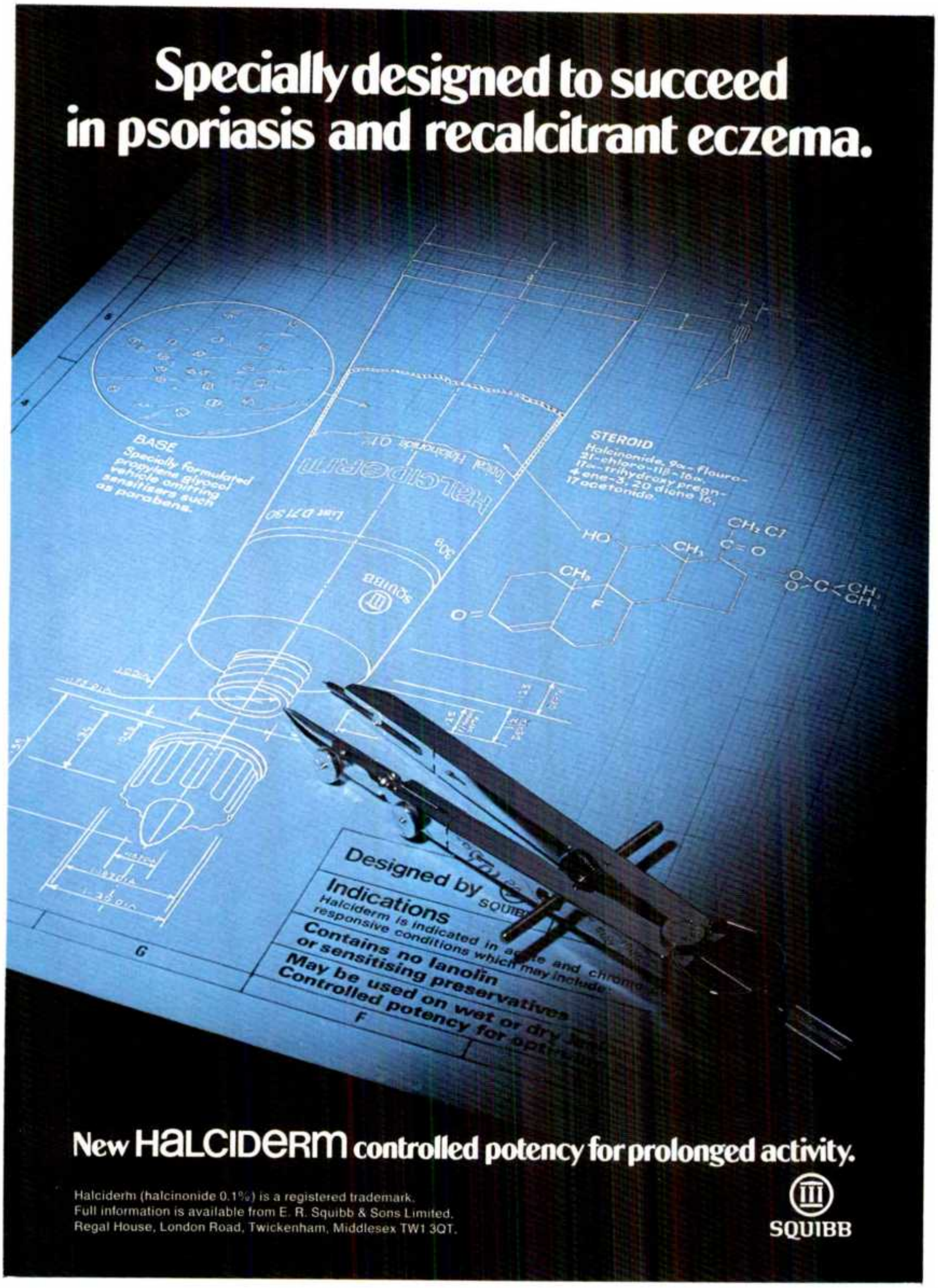
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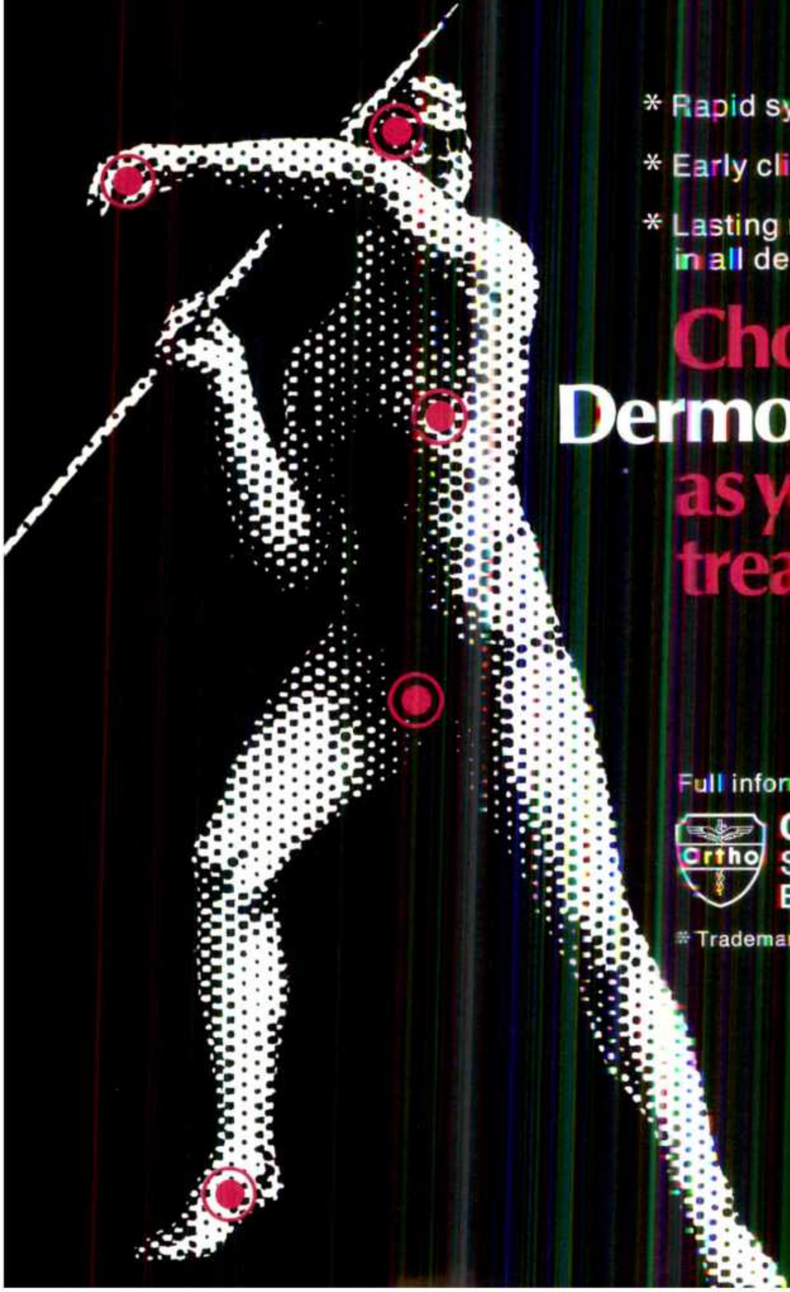

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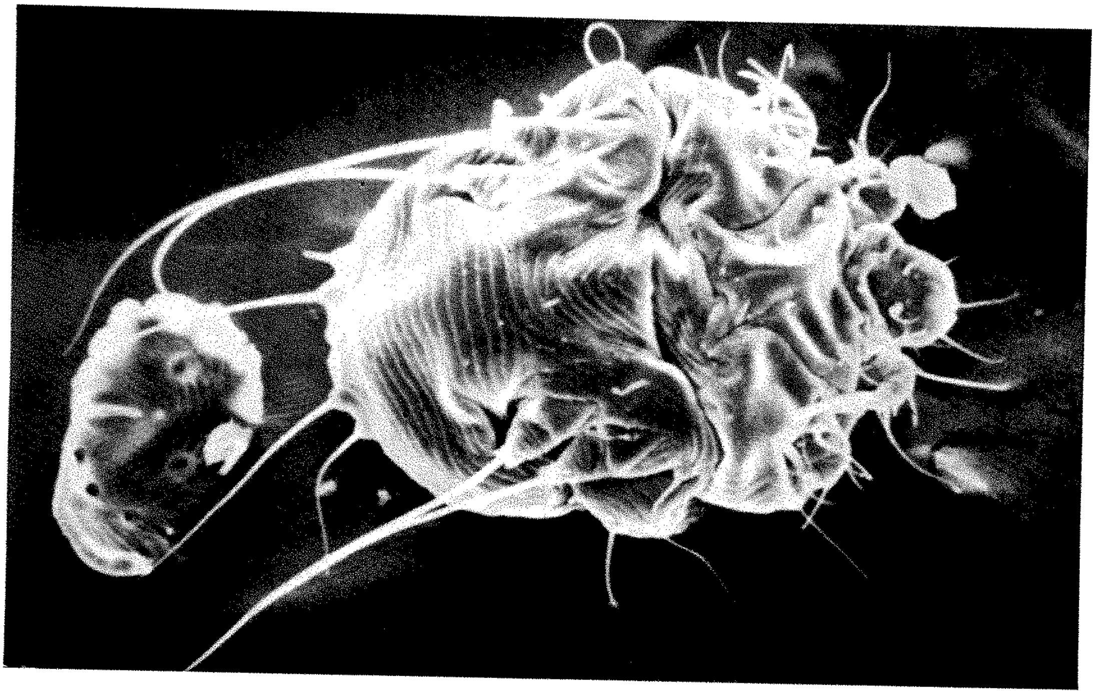

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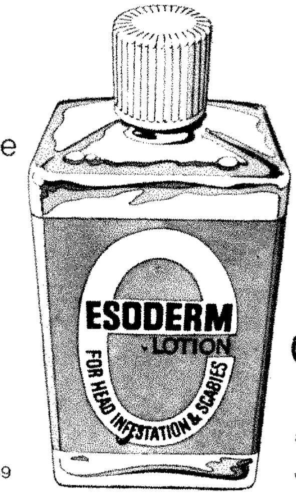

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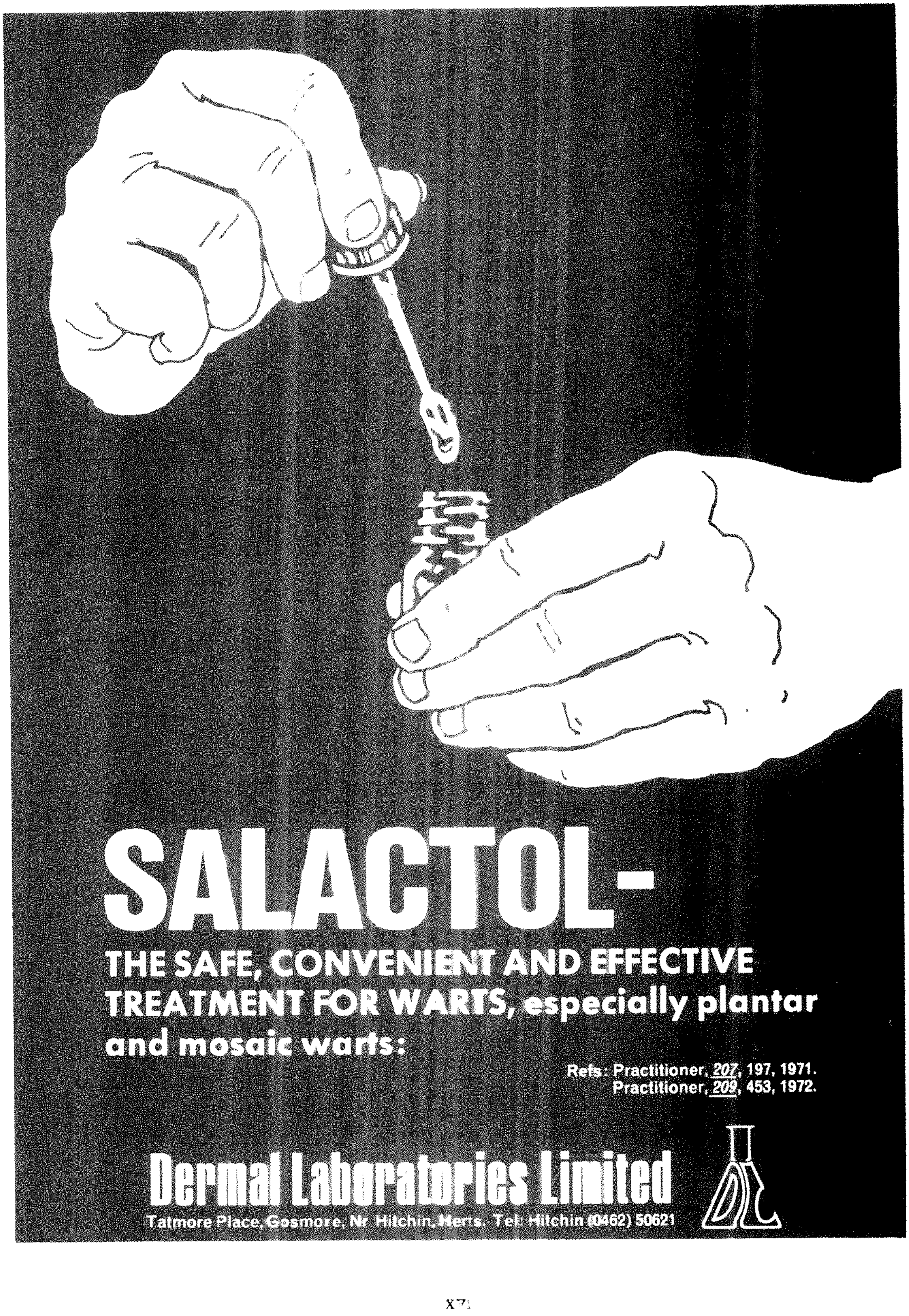

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British Journal of Dermatology (1975) **93**, 487.

Clinical and Laboratory Investigations

Natural antibiosis among skin bacteria as a primary defence against infection

SYDNEY SELWYN

Westminster Medical School, London SW1P 2AR

Accepted for publication 21 February 1975

SUMMARY

Antibiotic-producing bacteria were present on the healthy intact skin of 77 of 340 people but predominated in only 38·9% of these. In contrast, antibiotic-producers which occurred in the lesions of 51 out of 263 dermatological patients predominated in 78·4%. The presence of inhibitory commensals in skin lesions was associated with a significantly low incidence of secondary infection both on admission and during hospital stay; there was, however, a small excess of Gram-negative infections on admission in skin lesions containing antibiotic producers. Among ninety surgical patients, also, the presence of inhibitory skin bacteria was associated with a relatively low rate of wound colonization by pathogenic bacteria.

The ecological implications and practical applications of the findings are discussed.

In contrast to the modern enthusiasm for studying and administering antibiotic extracts, natural antibiosis in man has been almost completely ignored. The recent finding that antibiotic-producing commensals are present on the normal skin of over 20% of people (Selwyn & Ellis, 1972) raises the question of the possible value of such bacteria to their human hosts. An answer has been sought by determining the incidence of secondary infection in the dermatological lesions or surgical wounds of patients with and without such organisms. In addition, the ability of antibiotic producers to predominate over other bacteria on normal skin was also studied.

MATERIALS AND METHODS

Patients and normal subjects

The dermatological patients were in two groups: a retrospective series involving freeze-dried cultures from 233 patients originally described in a study of bacterial dispersal in hospital (Selwyn & Chalmers, 1965), and a further series of thirty recent in-patients. A typical skin lesion was swabbed in each case immediately after admission and before discharge. In the smaller series an apparently healthy skin area was also examined on these occasions.

The surgical series consisted of ninety patients undergoing planned operations on the abdomen or breast. Swabs moistened in Triton X-100 were vigorously rubbed over the operation site immediately

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before skin disinfection and incision. Swabs of the suture line were taken on the 7th post-operative day (and also earlier if sepsis was suspected).

The healthy skin flora of seventy medical students and 180 members of the hospital and laboratory staff was also studied to determine the relative predominance of antagonistic commensals.

Bacteriology

All swabs were cultured on nutrient agar containing 0·5% Tween 80 and 0·5% glucose at pH 6·5. Cultures were incubated aerobically at 33° C for at least 48 h unless spreading growth was noted after 18 h.

Representative strains of skin bacteria (including stored freeze-dried cultures from the retrospective series) were tested for inhibitory activity against indicator micrococci and against diphtheroid bacilli by means of the direct antagonism procedure described previously (Selwyn & Ellis, 1972). The freeze-dried strains appeared unharmed by their long storage. Only those organisms that produced clear inhibition zones extending at least 2 mm beyond the edge of the antagonistic colony are described as 'antibiotic producers'. These strains produced considerably wider zones in the deferred antagonism test, using a similar technique to that of bacteriocine typing.

All prevalent strains of *Micrococcaceae* and *diphtheroids* were identified by the methods of Baird-Parker (1965) and Evans (1968) respectively.

RESULTS

Normal skin

Commensals producing significant inhibition zones were present in fifty-six of the 250 students and staff, and in twenty-one of the ninety surgical patients pre-operatively (22·6% overall). As judged by the proportions of the different colony types in primary cultures, antibiotic-producers were truly predominant in only thirty of the seventy-seven positive cases (38·9%). Screening tests on sub-cultures were not always necessary for the detection of bacteria with inhibitory activity (Fig. 1). Resistance to the action of inhibitory organisms was uncommon in the accompanying flora.

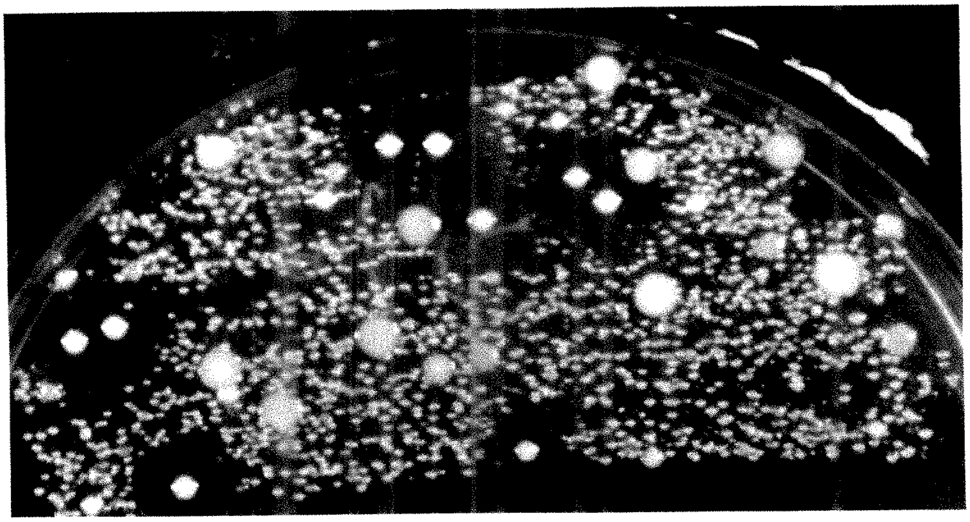

FIGURE 1. Primary culture of a skin swab showing inhibitory zones around a minority member of the flora (*Micrococcus* type 3).

Dermatological lesions

On admission, antibiotic producers and potential pathogens were cultured from skin lesions as shown in Table 1. The presence of inhibitory commensals was associated with a relatively low rate of secondary infection. However, the apparently protective effect of antibiotic producers was even more impressive in relation to infection by pathogens after hospital admission (Table 2). But protection did not extend to Gram-negative infections; indeed their incidence (9·9%) was somewhat higher in lesions possessing inhibitory organisms than in the absence of inhibitors (2·8% on admission and 6·1% subsequently).

TABLE 1. Inhibitory bacteria and infection in skin lesions on admission

		Patients with inhibitors		Patients without inhibitors	
Group	Total in group	Total no.	No. infected*	Total	No. infected*
I	233	42	8 (5)	191	76 (6)
II	30	9	0 (0)	21	8 (0)
Sum totals	263	51†	8 (5)‡	212	84 (6)

* Predominant Gram-negative cultures in parentheses.

† Inverse correlation of inhibitors and all pathogenic cultures: $\chi^2 = 9.33$; $P = 0.002$.

‡ Association between inhibitors in eight infected patients and Gram-negative cultures in five, compared with Gram-negative cultures in six out of eighty-four infections without inhibitors: $\chi^2 = 16{\cdot}33$; $P = 0{\cdot}001$.

Association between inhibitors in all fifty-one patients and Gram-negative cultures in five, compared with Gram-negative cultures in six out of 212 without inhibitors: $\chi^2 = 3{\cdot}40$; $P = 0{\cdot}04$.

Antibiotic producers when present in lesions were the predominant members of the flora in most cases, especially in eczema lesions and varicose ulcers (Table 3). Inhibitors were less often predominant in psoriasis. The normal areas of skin tested in group II yielded inhibitory commensals that were predominant in only four of nine patients, as compared with seven of nine corresponding skin lesions.

TABLE 2. Inhibitory bacteria in skin lesions on admission and hospital-acquired infection

		Patients with inhibitors		Patients without inhibitors	
Group	Total in group	Total no.	Hospital infections*	Total no.	Hospital infections*
I	233	42	6 (4)	191	103 (11)
II	30	9	1 (1)	21	9 (3)
Sum totals	263	51†	7 (5)‡	212	112 (14)

* Predominant Gram-negative cultures in parentheses.

† Inverse correlation of inhibitors and all pathogenic cultures; $\chi^2 = 27{\cdot}33$; $P < 0{\cdot}001$.

‡ Association between inhibitors in infected patients and Gram-negative cultures: $\chi^2 = 13{\cdot}86$; $P < 0{\cdot}001$.

Association between inhibitors in all patients and Gram-negative cultures: $\chi^2 = 0{\cdot}63$; $P = 0{\cdot}55$ (i.e. not significant).

Infections present on admission and acquired in hospital were mainly sub-clinical and chiefly involved *Staphylococcus aureus*, as previously described (Selwyn & Chalmers, 1965). In group I, three bacteriophage types of *Staph. aureus*, two bacteriocine types of *Pseudomonas aeruginosa* and a small number of strains of *Proteus mirabilis* accounted for most of the hospital infection.

TABLE 3. Inhibitory bacteria and hospital infection in various skin diseases

Diagnostic group	No.	Inhibitors present on admission*	Hospital infections in lesions	
			With inhibitors	Without inhibitors
Eczema	98	27 (24)	2	46
Psoriasis	91	14 (8)	4	25
Varicose ulcer	23	6 (5)	1	19
Besnier's prurigo	12	0	—	5
Acne	8	0	—	1
Drug eruptions	5	0	—	0
Mycosis fungoides	4	1 (1)	0	3
Pityriasis rosea	4	0	—	2
(Other conditions)	18	3 (2)	0	11
Sum totals	263	51 (40)	7	112

* No. of positive primary cultures with predominant inhibitors in parentheses.

Surgical wounds

Colonization of the suture line by potential pathogens occurred in two of the twenty-one patients who yielded inhibitors on admission, and in nineteen of the sixty-nine patients who did not possess such bacteria. The difference was, however, of 'no statistical significance', in contrast to that in the dermatological patients ($\chi^2 = 2\cdot 00$, $P = 0\cdot 16$). Only three clinical wound infections occurred. These were in patients without inhibitory bacteria; two were due to *Staph. aureus* and one to a coliform. Of the remaining eighteen cases of colonization by pathogens, *Staph. aureus* accounted for eleven and Gram-negative bacilli for seven (one being in a patient with inhibitory bacteria).

A strain of *Staph. aureus* that was prevalent in one of the surgical wards during part of the study was resistant to methicillin (mic 100 μg/ml), fusidic acid (mic 10 μg/ml) and gentamicin (mic 10 μg/ml), as well as to erythromycin, tetracycline, chloramphenicol and lincomycin. This organism was nevertheless highly sensitive to antibiotics produced by typical commensal cocci (Fig. 2).

Wounds were also cultured on the third day in twenty patients. Ten of these were suspected of having early wound infection, and ten others were tested at random. Five out of six patients who had inhibitory bacteria on admission yielded predominant cultures of these organisms post-operatively, compared with two of the six at the time of admission. *Staph. aureus* was prevalent in eight wounds, two of which became clinically infected; Gram-negative bacilli were predominant in three wounds, one of which became clinically infected. None of the eleven patients who were colonized by potential pathogens had inhibitory commensals on admission.

In all, 139 strains of inhibitory organisms were isolated from the dermatological and surgical patients and from the healthy subjects. All these strains belonged to the *Micrococcaceae*; seventy-two were assigned to the *Staphylococcus* genus and the remaining sixty-seven to the *Micrococcus* genus. Eleven individuals yielded two different inhibitory strains.

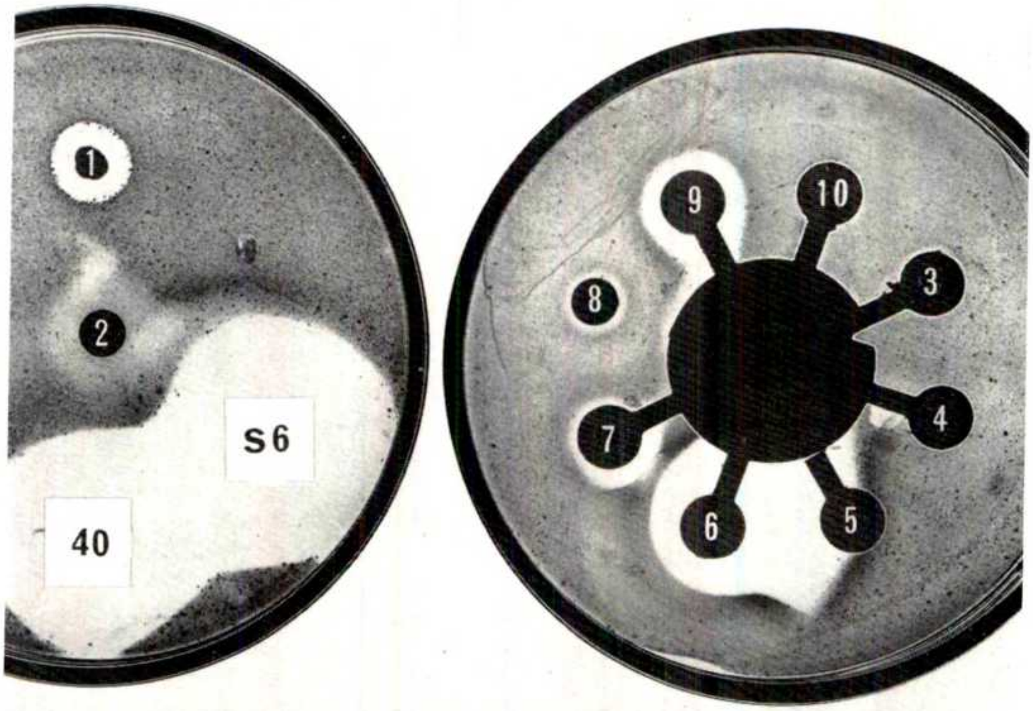

FIGURE 2. Sensitivity of a prevalent *Staph. aureus* strain on 5% salt agar to direct antagonism (1) and deferred antagonism ('S6' and '40') by commensals; resistance seen to methicillin (2), erythromycin (3), lincomycin (4), tetracycline (5), fusidic acid (6), neomycin (7), chloramphenicol (8), gentamicin (9), and benzylpenicillin (10). The discs contained 10 μg of each antibiotic except for benzylpenicillin (1·5 units), chloramphenicol (25 μg) and lincomycin (2 μg).

DISCUSSION

On healthy intact skin, commensals seem to derive no appreciable benefit from their ability to produce antibiotics. If they did have an ecological advantage over other commensals on normal skin they would have become predominant in all cases where resistant bacteria were not also present, instead of in less than half of the cases. Moreover, inhibitory strains would presumably have colonized everyone by now, rather than merely one-fifth of the population.

Healthy skin must undoubtedly provide very poor opportunities for bacteria to exploit their antagonistic abilities. In this relatively dry environment the diffusion of small amounts of antibiotic is probably minimal, especially in a mixed bacterial population that is comparatively sparse, scattered and multiplying at a very low rate. In these conditions antibiotic-producing organisms may even be under a relative disadvantage, due perhaps to a degree of auto-inhibition. Certainly, when antibiotic-producing skin commensals are grown *in vitro* in the presence of purified samples of their antibiotic, a moderate degree of inhibition takes place (unpublished observations).

In the analogous ecosystem of the soil, members of the *Streptomyces* group—despite their extraordinary capabilities as producers of broad-spectrum antibiotics—have fortunately not replaced the numerous micro-organisms involved in the nitrogen cycle and other vitally important natural processes (Gray & Williams, 1971). Indeed, the incidence of significant antibiotic production in *Streptomyces* spp. from different soil samples is 20% (Waksman, 1945), which is remarkably similar to the percentage of positive skin cultures.

If the skin is diseased or damaged, inhibitory commensals then seem able to exploit their antagonistic abilities. They were predominant when in skin lesions, notably in eczema and varicose ulceration; and their presence in dermatological patients was associated with an impressive reduction in the incidence of secondary infection, both before admission and in hospital. Many kinds of skin lesions provide very favourable conditions for bacterial multiplication (Selwyn & Chalmers, 1965). Presumably antagonistic activity is promoted by the denser bacterial population together with increased moisture and other factors in lesions that favour the production and diffusion of antibiotics.

In healing surgical wounds at the 7th post-operative day conditions were evidently less favourable for antibiotic-producing organisms than in skin lesions. Although the presence of inhibitory commensals seemed to afford some protection against colonisation by pathogens, many more surgical patients must be studied to increase the statistical significance of such results. However, in the immediate post-operative phase the wound more closely resembles severe eczema or ulceration, with transudation of tissue fluids and serum, and increased susceptibility to infection. At this stage inhibitory bacteria predominate more readily and should provide a valuable defence. Future studies on surgical patients should therefore concentrate on this crucial period.

The possibility that antibiotic producers encourage Gram-negative infection is of great interest. Skin lesions on admission certainly showed a small but significant excess of Gram-negative colonizations in the presence of antibiotic producers. Indeed, the wide range over which these antibiotics act in mixed cultures notably excludes the *Enterobacteriaceae* and *Pseudomonas* groups (Selwyn & Ellis, 1972), though the purified antibiotics show some activity against those organisms (Selwyn & Sethna, in preparation). In this respect antibiotics from skin commensals resemble an agent such as hexachlorophane which has been held responsible for encouraging Gram-negative hospital infections (Forfar *et al.*, 1968). There is, however, an essential difference between natural antibiosis and the use of any agent that acts selectively on Gram-positive bacteria. The antibiotic-producing commensals remain prevalent on the skin, whereas selective agents suppress the entire accessible normal flora, so creating a potentially dangerous ecological disturbance. The risks of overgrowth by Gram-negative bacilli (or even fungi) are therefore theoretically far greater with a selective anti-bacterial agent than with naturally-occurring inhibitory bacteria. Certainly, there was no significant excess of Gram-negative infections among dermatological patients who possessed inhibitory bacteria while in hospital, despite very heavy environmental contamination with *Pseudomonas* and *Proteus* bacilli during the main part of the original study period (Selwyn, 1965).

Confirmation of the apparent defensive value of antibiotic-producing commensals should be sought in early surgical wounds, and cases with skin lacerations, as well as burns, which should provide an excellent model for further study. Clearly another approach is the direct investigation of the protection against infection afforded by the artificial colonization of such lesions with suitable commensals. Modern work in this field has usually involved—quite unaccountably—the inoculation of the pathogen *Staph. aureus*, and the most popular strain used ('502A') does not even produce demonstrable antibiotic (Anthony & Wannamaker, 1967). By contrast, in an age of increasing ecological enlightenment the harnessing of normal skin bacteria for the benefit of their host should prove deeply satisfying. Certainly the preliminary results of animal experiments involving the simultaneous implantation of *Staph. epidermidis* and *Staph. aureus* seem encouraging (Pryjma, Heczka & Wilburg, 1972).

ACKNOWLEDGMENTS

I wish to thank Miss Sussan Eftehari, Mr Derek Manson-Smith and Mrs Sheila Waite for their technical assistance at different stages of this work. I am grateful also to Mr David Brown of the Computer Centre, Westminster Hospital for statistical advice. Dr Peter Samman, Dr Peter Copeman and Professor Harold Ellis kindly allowed access to their patients at all times. Financial support for this investigation was provided by the Research Committee, Westminster Hospital and the Peel Medical Research Trust. A grant towards expenses was also provided by Glaxo Laboratories Ltd.

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Ultrastructural localization and differentiation of membrane-bound ATP utilizing enzymes including adenyl cyclase in normal and psoriatic epidermis*

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Accepted for publication 21 February 1975

SUMMARY

The total membrane-bound ATP hydrolytic activity in human epidermis is due to the activities of at least three differently located enzymes, namely Mg^{++}-activated ATPase, phosphomonoesterase and adenyl cyclase.

Cytochemical studies on psoriatic epidermis with various inhibitory and stimulatory substances showed reduced activities of ATPase and phosphomonoesterase, and a lack of sensitivity of adenyl cyclase to specific stimulators such as isoproterenol and glucagon. Since no differences of basal adenyl cyclase activity were observed between normal and psoriatic human skin without stimulation, it seems likely that in psoriasis a latent defect of adenyl cyclase may exist, resulting in a deficient response of this enzyme to regulatory agents.

In conclusion, the present study reveals that not a single enzyme but the entire membrane-bound nucleotide metabolism is altered in psoriatic keratinocytes, causing a disturbance of the membrane-bound energy utilization, similar to findings in proliferating tumour cells.

In previous investigations we showed by various techniques that in psoriatic keratinocytes alterations of the cell surface architecture occur (Orfanos *et al.*, 1973). These studies suggested a loss of contact inhibition of growth in psoriasis, as reported in virus transformed and in malignant fibroblasts *in vitro*. The intervening events between cell surface alterations and intracellular response, however, have remained obscure.

Membrane-bound enzymes are assumed to be involved in the regulation of growth as a mediator system; these transform extracellular information and influence intracellular metabolism (Robinson, Butcher & Sutherland, 1968; Otten, Johnson & Pastan, 1971). In proliferating psoriatic epidermis, changes of the membrane-bound enzymatic activity corresponding to the suggested loss of contact inhibition and the increased mitotic rate might therefore be anticipated. Recent cytochemical studies

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[*] Presented at the Second Annual Meeting of the Arbeitsgemeinschaft Dermatologische Forschung, Düsseldorf, 15–17 November, 1974.

have indeed revealed that the total activity of ATP hydrolytic enzymes is markedly reduced on the cell membranes of psoriatic keratinocytes (Mahrle & Orfanos, 1974).

In the present study we have investigated various membrane-bound ATP utilizing enzymes by the application of different substrates, appropriate inhibitors and stimulating agents. These investigations included an attempt to localize adenyl cyclase activity in normal and psoriatic epidermis.

MATERIAL AND METHODS

Four or six mm punch biopsies were taken from untreated psoriatic lesions of four patients and from the normal skin of four healthy volunteers under local anaesthesia with 1% Scandicain. The skin specimens were prefixed at 4°C in a fixative solution consisting of 4% paraformaldehyde, 2·5% glutaraldehyde and 0·05% $CaCl_2$, buffered with 0·1 mol/l cacodylate buffer at pH 7·2, for 4 h. The specimens were cut into 1·0 mm thick pieces during prefixation, sliced into 0·1 mm thick flakes and rinsed in cold cacodylate buffer. Incubation was then performed in the following media:

(1) In a modified Wachstein–Meisel medium for *ATP hydrolases* as described earlier (Mahrle & Orfanos, 1974); for further differentiation the following experiments were carried out: (a) incubation in standard medium plus 1 mmol/l ouabain; (b) incubation in standard medium after substituting ATP by 5 mmol/l Na-β-glycerophosphate, at pH 5·0 and pH 9·0; (c) incubation in standard medium after substituting ATP by 5 mmol/l AMP, at pH 7·2. The incubation time was 90 min at 37°C.

(2) In a medium described by Reik *et al.* (1970) for cytochemical localization of *adenyl cyclase*. This cytochemical assay is based on the precipitation of a lead pyrophosphate salt following the action of adenyl cyclase on ATP:

$$\mathrm{ATP}\xrightarrow[\text{cyclase}]{\text{adenyl}}\mathrm{cAMP}+\mathrm{PPi}$$

In this case theophylline was added to inhibit the phosphodiesterase activity and the release of phosphate in the reaction sequence:

$$\mathrm{cAMP}\xrightarrow[\text{diesterase}]{\text{phospho-}}5'\text{-AMP}\xrightarrow{5'\text{-nucleotidase}}\begin{array}{c}\text{adenosine}\\+\\\text{phosphate}\end{array}$$

The standard medium contained 0·5 mmol/l ATP, 2·0 mmol/l theophylline, 4·0 mmol/l $MgSO_4$, and 4·8 mmol/l $Pb(NO_3)_2$ in 80 mmol/l Tris-maleate buffer, at pH 7·4. The reaction was carried out for 30 min in a water bath at 30°C.

Additional experiments with this standard medium and under the same conditions were performed in presence of (a) $12{\cdot}5\times10^{-3}$ mol/l NaF, (b) $1{\cdot}5\times10^{-7}$ mol/l glucagon, (c) 4×10^{-6} mol/l l-isoproterenol, and (d) 4×10^{-6} mol/l l-isoproterenol and 1×10^{-3} mol/l propranolol.

After incubation all tissue flakes were rinsed in cold cacodylate buffer, fixed in 1% osmic acid solution buffered with acetate–Veronal buffer for 1 h at pH 7·2, dehydrated in rising concentrations of acetone, and were then embedded in Araldite®. Ultrathin sections were obtained and examined with an electron microscope before and after staining with uranyl acetate and lead citrate.

RESULTS

(1) *ATP hydrolases*

Findings regarding *ATP hydrolytic activity* of normal and psoriatic epidermis have been reported

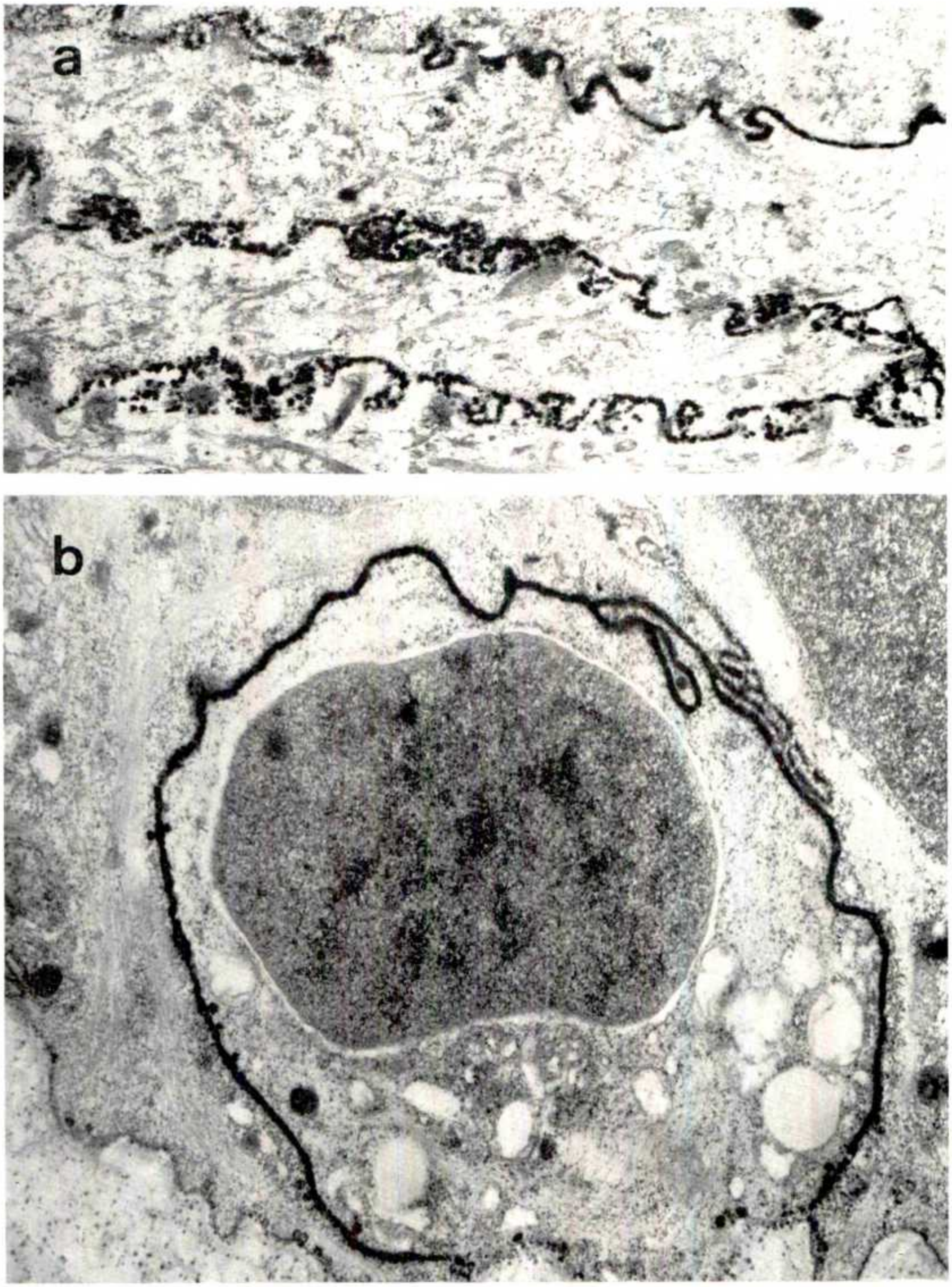

FIGURE 1. In normal epidermis the ATPase reaction (5 mmol/l ATP, pH 7·2) is not inhibited by 1 mmol/l ouabain: (a) on the cell surfaces in the upper epidermis (× 21,000), (b) on the surface of what is probably a melanocyte in the basal epidermal layer (× 17,500).

extensively elsewhere (Mahrle & Orfanos, 1974) and are limited here to our observations on the epidermal cell membranes. In normal epidermis we found a strong ATP hydrolytic activity localized on the cell membranes and in the intercellular spaces of the stratum granulosum. The activity decreased to the lower layers of the stratum Malpighii where reaction products faintly marked the cell membrane of the keratinocytes. A distinctly positive reaction was also observed in the basal epidermal layer on the cell membranes facing the dermis.

In psoriatic epidermis a similar reaction was seen on the cell membranes and in the intercellular spaces. However, the dense deposits failed to form a continuous layer and large areas of the cell membranes remained unstained.

The present investigation showed that addition of *ouabain* to the incubation medium did not produce any change in the amount of reaction products on the cell surfaces in either normal or psoriatic epidermis (Fig. 1a, b).

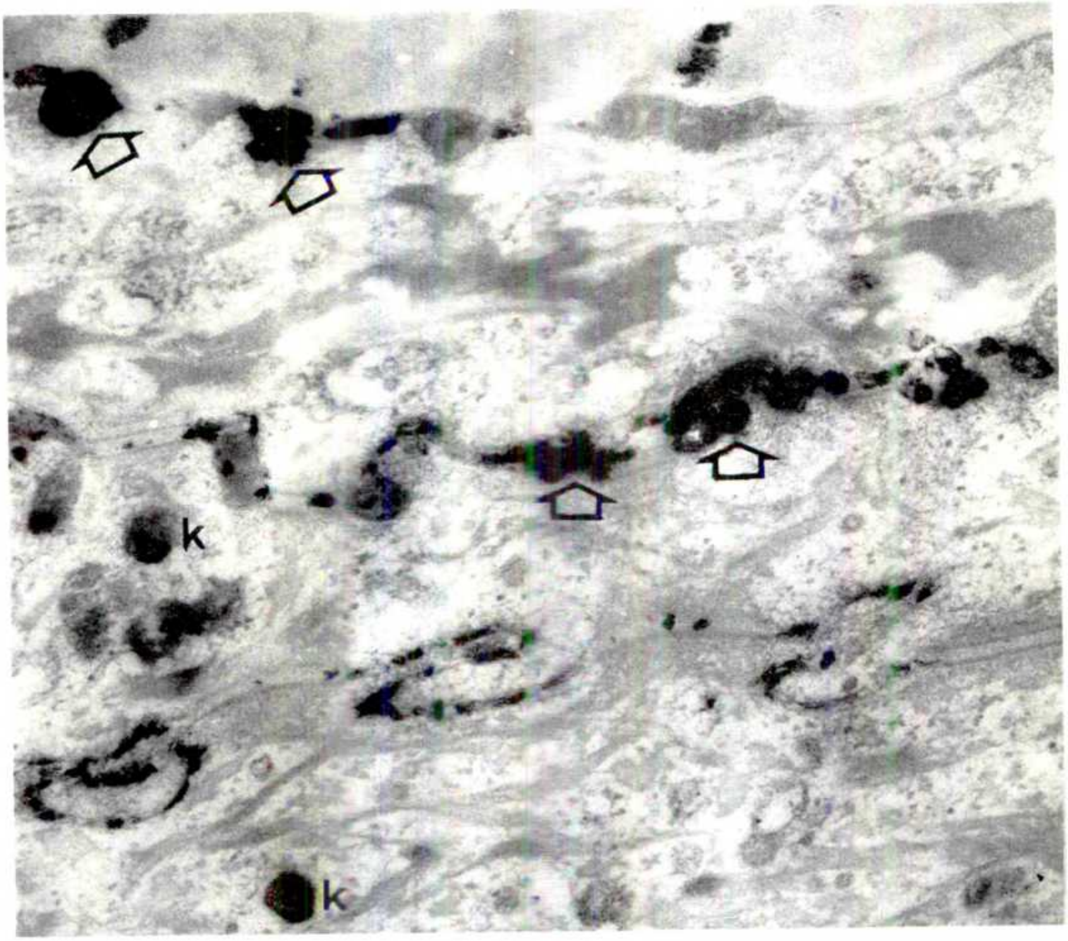

FIGURE 2. Using 5 mmol/l Na-β-glycerophosphate (pH 5·0) as substrate electron dense reaction products are situated in the intercellular spaces of normal stratum granulosum and intermedium. Salt deposits are partly bound to cytoplasmic keratinosomes (k) and to fragments of keratinosomes in the intercellular space (arrows) (× 57,000).

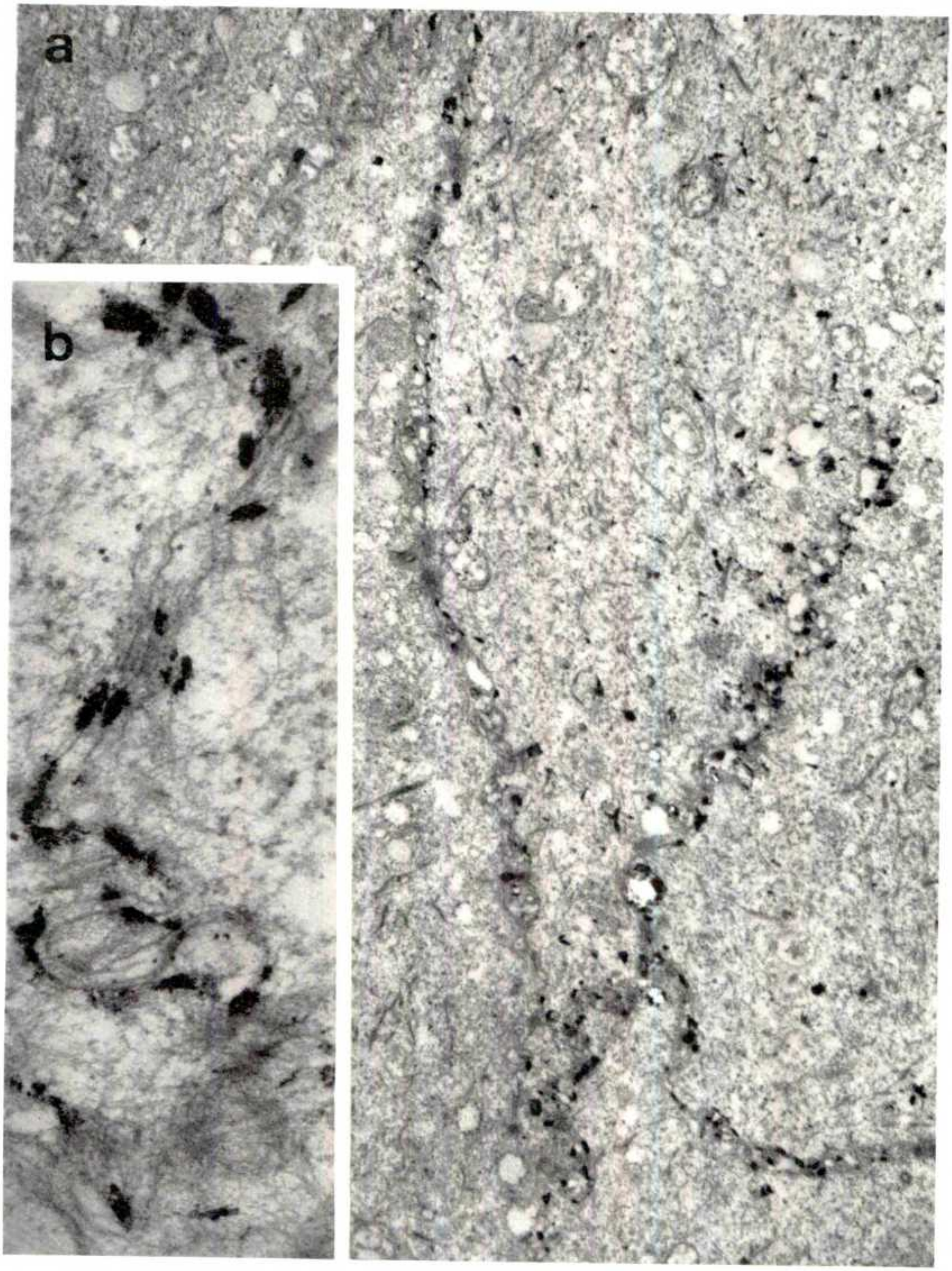

FIGURE 3. In the stratum intermedium of psoriatic epidermis the amount of acid phosphomonoesterase reaction products is relatively reduced; (a) on the cell membranes (× 15,200), and (b) more sporadically distributed than in normal epidermis (× 57,000).

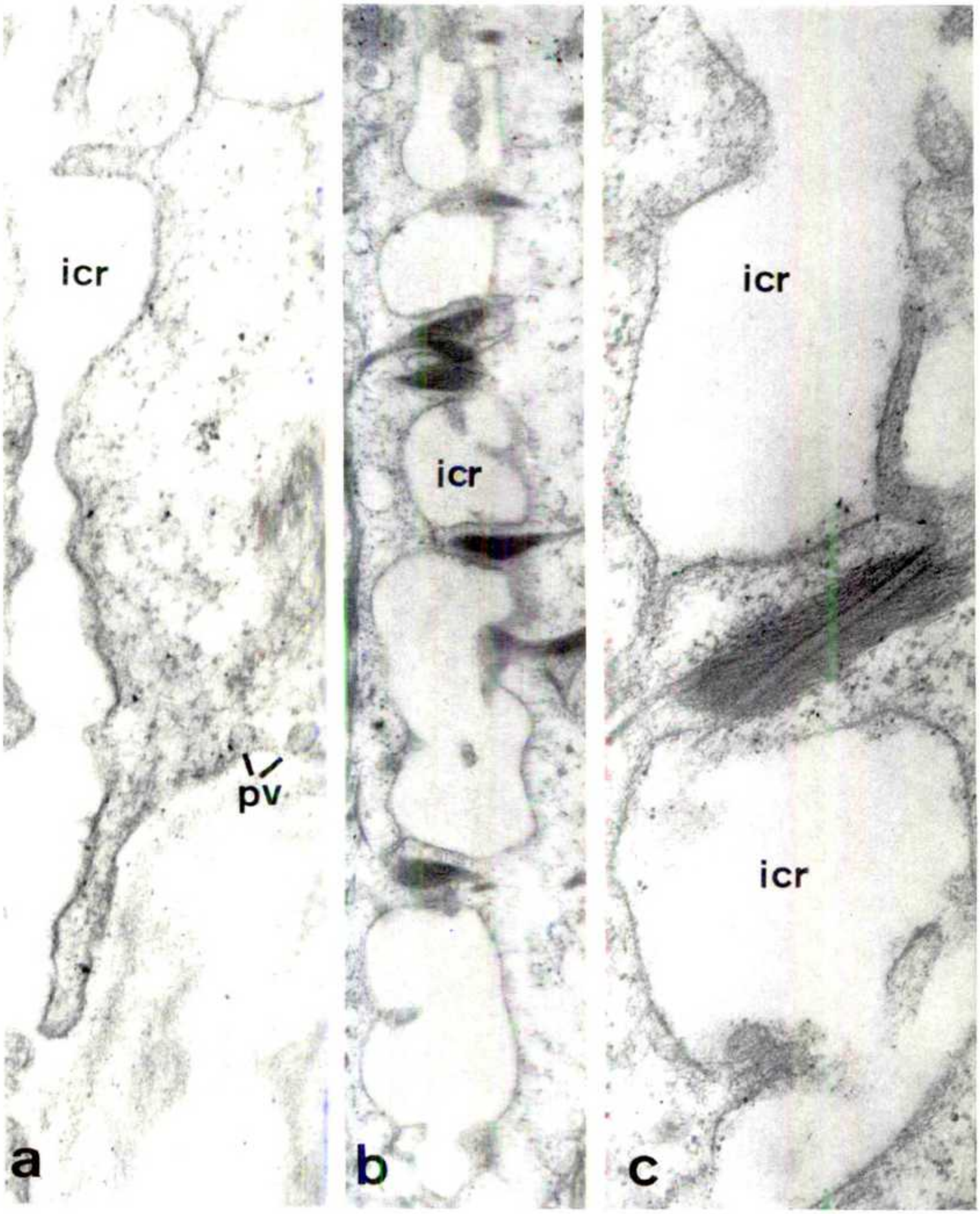

FIGURE 4. Adenyl cyclase reaction (0·5 mmol/l ATP, 2·0 mmol/l theophylline) without stimulation reveals no reaction products on the cell membranes, in the intercellular spaces (ics), or in the pinocytotic vesicles of basal cells (pv) in (a) normal, and (c) in psoriatic epidermis. (b) Isoproterenol stimulated adenyl cyclase reaction in normal epidermis (compare Fig. 7) is inhibited by 10^{-3} mol/l propranolol (Magnification: (a) and (c) × 57,000, (b) × 29,000).

Using *Na-β-glycerophosphate* as substrate at pH 5·0 the reaction product was limited to the granular layer and to the transitional zone of normal epidermis (Fig. 2). The salt deposits were partly splintery shaped or needle-like and were seemingly attached to cytoplasmic keratinosomes and/or fragments of keratinosomes in the intercellular space. Some electron-dense deposits were also found in the basal layer on the cell membranes facing the dermis. At pH 9·0 no reaction product was seen in the epidermis of any specimen.

In psoriatic epidermis Na-β-glycerophosphate hydrolysis occurred at pH 5·0 on the cell membrane and in the intercellular spaces of the entire stratum intermedium and extended into the stratum corneum (Fig. 3a, b). There was a broad reaction zone in psoriatic epidermis compared to normal; however, the relative amount of lead deposits on the surface of psoriatic keratinocytes was definitely reduced. In the basal layer of psoriatic epidermis the cell membranes facing the dermis failed to stain. Only faint and intermittent deposits occurred in some areas. At 9·0 pH no reaction products were seen, as in the case of normal epidermis.

When *AMP* was added as substrate, small deposits were only occasionally present in the intercellular spaces of the upper epidermis, indicating a low 5′-nucleotidase activity. No significant difference was seen between normal and psoriatic epidermis under these conditions.

(2) *Adenyl cyclase*

Normal and psoriatic epidermis incubated in Reik's medium for adenyl cyclase revealed little or no activity under the electron microscope (Fig. 4a, c). Deposits of reaction product were rarely accumulated on the surface of keratinocytes in the upper cell layers. Addition of *NaF* to the incubation medium had no significant influence on the pattern and the intensity of the reaction.

In the presence of $1 \cdot 5 \times 10^{-7}$ mol/l *glucagon* an increase of the amount of reaction products occurred on the surface of normal keratinocytes in the upper epidermal cell layers. In addition, we found a clearly positive reaction in the lower parts of normal epidermis and in the basal layer, which was not seen without glucagon (Figs 5 and 6a–c). The whole procedure was repeated a number of times and several tissue blocks were sampled from each experiment for confirmation. In contrast to the ATP reaction, the electron dense deposits in the basal layer seemed to be confined mainly to the lateral and epidermal aspects of the basal cells, including melanocytes, whereas deposits on the membranes facing the dermis were only rarely encountered.

Incubation with 4×10^{-6} mol/l *l-isoproterenol* also produced an increase of the surface-bound and the intercellular deposits in normal epidermis (Fig. 7a–c). In some areas the reaction product was even found in the intercellular spaces of the lower layers of the stratum corneum. In addition, there was an enhancement of the reaction in some regions of the basal epidermal layer, especially at the dermal facing membranes. Addition of 1×10^{-3} mol/l *propranolol* into the incubation medium clearly had an inhibitory effect on isoproterenol stimulation, since little or no reaction product was found in the intercellular spaces or elsewhere under these conditions (Fig. 4b). In psoriatic epidermis, on the other hand, we were not able to detect any alteration of the intensity and the distribution pattern of the reaction product following the addition of glucagon, isoproterenol and/or propranolol to the incubation medium.

COMMENT

The evidence presented in this study confirms that the total membrane-bound ATP hydrolytic activity is significantly reduced in psoriatic epidermis. Although non-enzymatic hydrolysis cannot be completely neglected using histochemical lead methods for the demonstration of ATP hydrolases

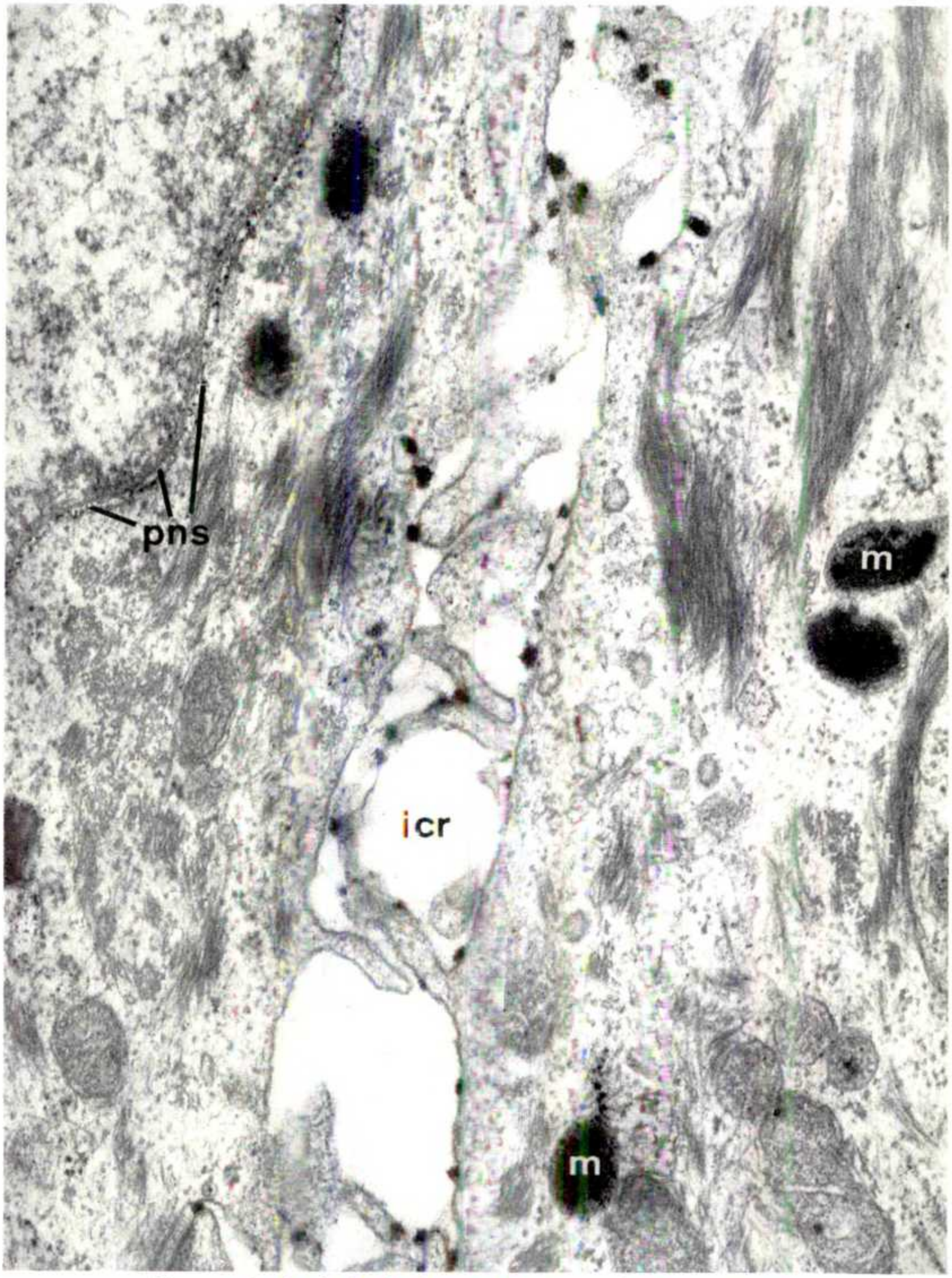

FIGURE 5. Glucagon stimulated adenyl cyclase reaction ($1{\cdot}5 \times 10^{-7}$ mol/l glucagon). Focal deposits are seen at the cell surfaces in the deeper layers of normal epidermis. Melanosomes (m) and perinuclear space (pns) show nonspecific staining (× 57,000).

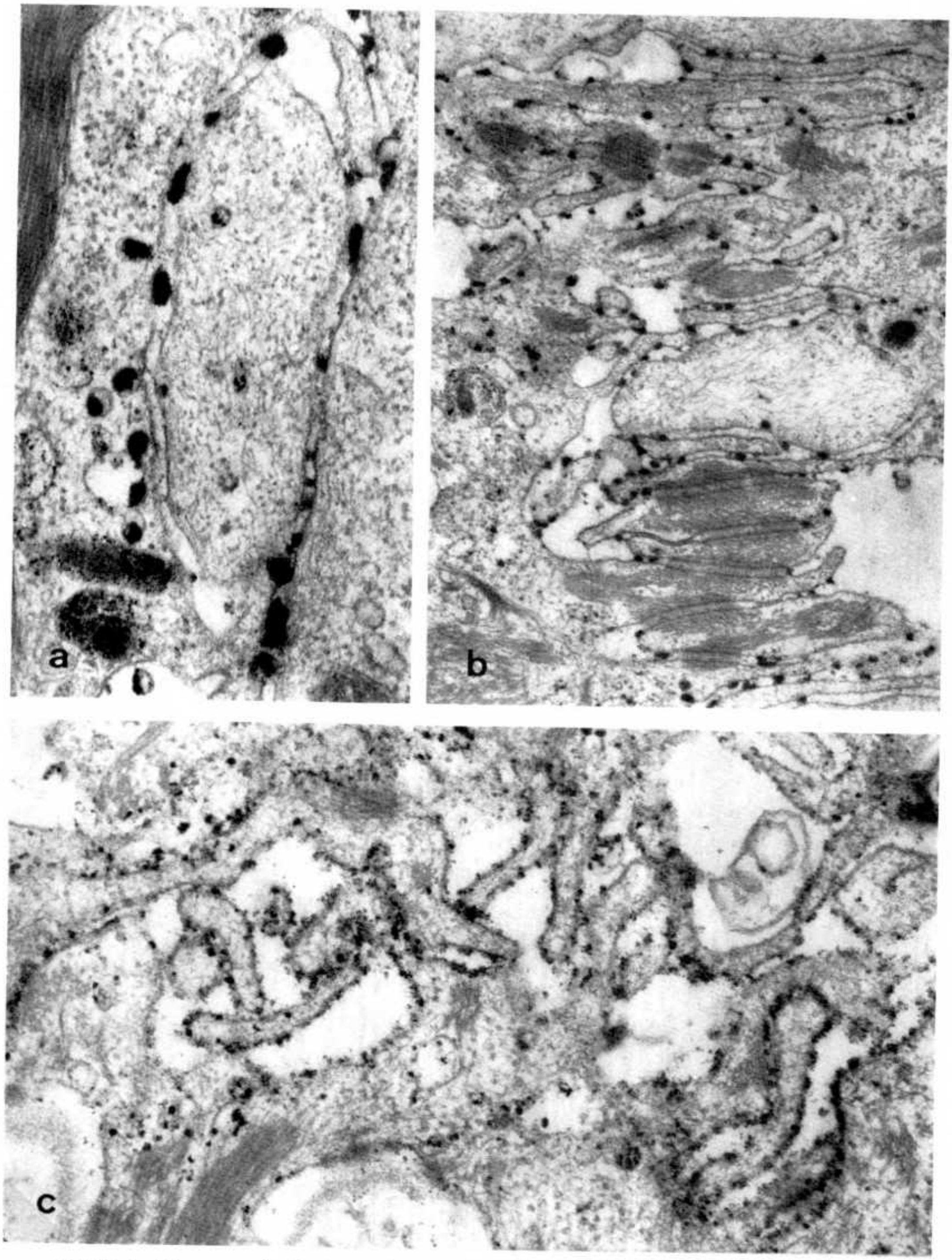

FIGURE 6. Glucagon stimulated membrane-bound adenyl cyclase reaction deposits in (b) the deeper layers, and (a, c) in the basal layer of normal epidermis (Magnification: a and c × 57,000, b × 36,000).

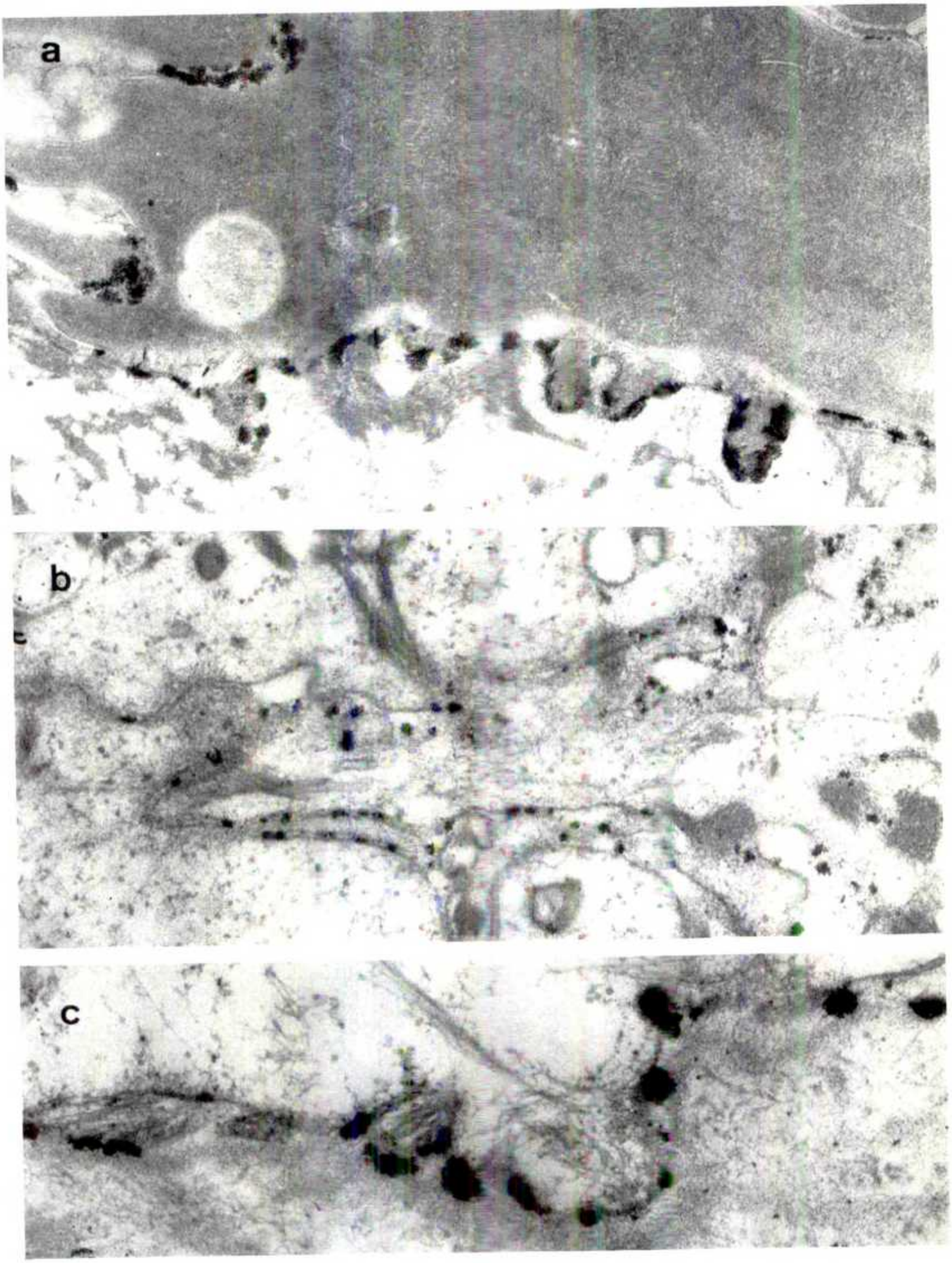

FIGURE 7. Isoproterenol stimulated adenyl cyclase reaction (4×10^{-6} mol/l l-isoproterenol) in normal epidermis. (a) Stratum intermedium and corneum, (b) deeper epidermal layers, (c) dermal–epidermal interface (Magnification: all ×57 000).

(Keonig & Vial, 1973), we feel that hydrolases splitting energy-rich bonds of β- and γ-phosphate residues are responsible for the reaction product under the experimental conditions employed.

Farquhar & Palade (1966) demonstrated *ATPase activity* in frog and toad epidermis with the same technique. However, Mg^{++}-activated membrane-bound ATPase in the skin of these animals was found to be insensitive to ouabain, similar to the present data in human skin. For this reason further differentiation of epidermal ATP hydrolytic activity was necessary, since a number of enzymes including phosphomonoesterases, nucleotidases, pyro- and polyphosphatases are able to hydrolyse ATP (Wolff, 1964; Ishikawa & Klingmüller, 1964). Our present results indicate that AMP hydrolysing enzymes, such as *5'-nucleotidase*, show a low activity in normal human and in psoriatic epidermis. On the other hand, this investigation reveals a strong *acid phosphomonoesterase* activity in the intercellular spaces of the granular layer, hydrolysing Na-β-glycerophosphate at pH 5·0, whereas the activity of phosphomonoesterase is minimal or absent at pH 9·0. Phosphomonoesterases, therefore, may contribute in some degree to the utilization of ATP in the upper layers of human epidermis. Admittedly, the activities of different hydrolases overlap at this point (Montagna & Ellis, 1962).

We have also demonstrated that *adenyl cyclase* (catalysing cAMP formation) utilizes ATP at the cell membranes. Because of the paucity of cytochemical information on this point, we used an ultrastructural technique, which has been successfully applied in liver cells, Langerhans islet cells and endothelial cells (Reik *et al.*, 1970; Howell & Whitfield, 1972; Wollenberger, Schulze & Krause, 1973). However, little or no activity of adenyl cyclase was found in human skin without stimulation, probably because adenyl cyclase is less active in epidermal cells than in liver cells (Mier & Urselmann, 1970). The addition of fluoride had no stimulatory effect on adenyl cyclase in intact human epidermis and may do so only in broken cells (Wright *et al.*, 1973). Nevertheless, using other specific stimulatory agents such as glucagon (Mier & Urselmann, 1970) and isoproterenol (Duell *et al.*, 1971) we found additional electron-dense deposits at the cell surfaces in the upper layers and, more distinctly, in the lower layers of normal epidermis. Since catecholamines stimulate adenyl cyclase activity without stimulation of other ATP utilizing enzymes (Katz & Tenenhouse, 1973), this cytochemical reaction was considered to be due to adenyl cyclase activity in these regions. Moreover, the inhibitory effect of propranolol on the isoproterenol stimulation provided strong evidence supporting this suggestion.

Summarizing these findings, it seems obvious that the total membrane-bound ATP hydrolytic activity in human epidermis is a sum of the activities of at least three different membrane-bound enzymes, namely Mg^{++}-activated ATPase, phosphomonoesterase and adenyl cyclase. The participation of each enzyme in ATP utilization differs according to the epidermal level, the Mg^{++}-activated ATPase and the glucagon- and isoproterenol-stimulated adenyl cyclase activity being located mainly in the upper and lower epidermal layers and the phosphomonoesterase activity being located almost exclusively in the granular layer. Accordingly, the decreased total ATP hydrolytic activity in psoriatic epidermis may be the result of the reduced activities of Mg^{++}-activated ATPase and phosphomonoesterase and of the lack of sensitivity of adenyl cyclase to specific stimulators.

Biochemical data on adenyl cyclase activity in psoriasis are contradictory (Hsia *et al.*, 1972; Härkönen, Hopsu-Havu & Ray, 1974). The present results confirm and extend the biochemical observations of Wright *et al.* (1973), who found that in psoriatic plaques the adenyl cyclase was unresponsive to NaF and less responsive than normally to adrenaline. In agreement with the data of Härkönen *et al.* (1974) our findings do not provide any indication of an altered basal activity of adenyl cyclase in psoriasis without stimulation. It seems likely, however, that in psoriasis a latent defect of the adenyl cyclase exists, resulting in a deficient response of this enzyme to regulatory substances. The possible influence and alteration of receptor sites for regulatory agents on the cell surface of psoriatic keratinocytes needs further investigation.

Thus our findings imply that the diminished ATP utilization at the cell membranes in psoriasis is not confined to a possible defect of a single enzyme. On the contrary, it seems that the entire membrane-bound adenine nucleotide metabolism is altered. Since adenine nucleotides are powerful modulators of key regulatory enzymes, such a disturbance of the membrane-bound energy utilization would have a major significance in psoriasis.

Interestingly enough, alterations of the membrane-bound ATP hydrolases and adenyl cyclase have been reported in the cells of some tumours (Sugár, Csuka & Tóth, 1968; Agren *et al.*, 1971; Emmelot & Bos, 1971; Makman, 1971; Karasaki, 1972), similar to those in proliferating psoriatic epidermis described in this study. In both benign and malignant proliferation functional membrane defects correspond to the loss of glycocalyx and to the structural alterations of the cell membranes (Burger & Noonan, 1970; Orfanos *et al.*, 1973). In this context it is important to note that lipid and protein components of the cell membrane and their compartmentation influence the activity of membrane-bound enzymes (Grisham & Barnett, 1972; Sood, Sweet & Zull, 1972; Redwood, Gibbes & Thompson, 1973; Roelofsen & Van Deenen, 1973). Thus an isolated alteration of a membrane component may lead to a general functional membrane defect and to a disturbance of energy utilization in the involved tissue.

ACKNOWLEDGMENT

We should like to thank Mrs E.Bodeux and Mrs I.Westermann for their valuable technical assistance.

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Lysosomal hydrolases of the epidermis

3. PEPTIDE HYDROLASES

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Accepted for publication 10 January 1975

SUMMARY

Four distinct peptide hydrolases (EC 3·4) have been characterized in guinea-pig epidermis; these are cathepsin B_1, cathepsin C, cathepsin D and arylamidase. Their properties are consistent with those of lysosomal enzymes. Cathepsin E was not detected.

The peptide hydrolases (EC 3·4) may be divided into the exopeptidases (such as cathepsins A and C), which remove terminal amino acids from polypeptide chains, and the endopeptidases (including cathepsins D and E, the 'neutral' proteinases, collagenase, etc.) which cleave protein molecules internally. Certain enzymes, such as cathepsin B_1, possess both exo- and endopeptidase activity. The arylamidases (amino acid naphthylamidases) are commonly regarded as peptide hydrolases, although their normal physiological substrate is still unknown.

A great deal of miscellaneous information has accumulated regarding the peptidase activity of whole skin (reviewed by Hopsu-Havu & Jansén, 1969a); some studies of isolated epidermis have also been reported. Unfortunately, much of the earlier literature is difficult to interpret because classification of the peptide hydrolases has been unsatisfactory and many of the individual enzymes were, until recently, ill-defined. A number of more or less specific assays for the lysosomal peptidases are now available, however (Barrett, 1972); we have adapted certain of these to characterize and quantify peptidases in guinea-pig epidermis. Our results are presented in this communication.

MATERIALS

Fluorogenic substrates used in this investigation are listed in Tables 1 and 3. The amino acid naphthylamides were purchased from Sigma Chemical Co. Ltd.; all protein–fluorescein conjugates were prepared as described by de Lumen & Tappel (1970) for haemoglobin, and were purified by chromatography over Sephadex G-25.

All other materials were as described previously (Mier & van den Hurk, 1975).

TABLE 1. Assay systems used for the continuous fluorimetric measurement of cathepsin B_1, cathepsin C and arylamidase. Substrates were added as solutions in dimethyl sulphoxide (100, 10 and 10 mmol/l respectively). The total reaction volume was 0·5 ml. In the chloride-dependence studies, cysteamine HCl was replaced by cysteine (free base)

EC number	Enzyme	Composition of assay system					
		Substrate (μmol)	Phosphate (μmol)	EDTA (μmol)	Cysteamine HCl (μmol)	Epidermis (mg equiv.)	pH
3.4.4.–	Cathepsin B_1	α-N-benzoyl-DL-arginine β-naphthylamide HCl (1·0)	25·0	0·3	1·0	0·5	6·4
3.4.4.9	Cathepsin C	glycyl-L-phenylalanine β-naphthylamide (0·1)	25·0	0·3	1·0	0·2	5·0
3.5.1.–	Arylamidase	L-leucyl-β-naphthylamide HCl (0·1)	25·0	—	—	0·2	7·8

METHODS

Assay procedures

Cathepsin B_1, cathepsin C and arylamidase. Assay was based on the fluorimetric determination of the 2-naphthylamine (NA) released from appropriate substrates. Both continuous and stop-time methods were used, the former being employed for the characterization of individual enzymes and the latter for the simultaneous quantitation of different enzymes ('normal values').

For continuous assay, measurements were carried out at 25 °C using a total reaction volume of 0·5 ml; the compositions of the reaction mixtures are shown in Table 1. Changes in fluorescence were followed using a 'Fluorispec' spectrophotofluorimeter (Baird Atomic) fitted with a 'Servogor' flat bed recorder ($\lambda_{ex} = 336$, $\lambda_{em} = 414$ nm). In all cases the reaction was allowed to proceed until linearity was established (2–15 min). The system was calibrated using a standard solution of NA (2×10^{-6} mol/l) in the appropriate buffer, and enzyme activity calculated as nmol (10^{-9} mol) of NA liberated per min per g fresh weight of epidermis.

TABLE 2. Stop-time assay systems for cathepsin B_1, cathepsin C and arylamidase. Reaction was initiated by the addition of 0·1 ml epidermal extract to 0·1 ml buffered substrate. Substrates were dissolved in dimethyl sulphoxide as before

Enzyme	Concentration of epidermal extract (mg/ml)	Composition of buffered substrate (mmol/l)				
		Substrate	Phosphate	EDTA	Cysteamine HCl	pH
Cathepsin B_1	5	α-N-benzoyl-DL-arginine β-naphthylamide HCl (2·0)	100	1·2	4·0	6·4
Cathepsin C	2	glycyl-L-phenylalanine β-naphthylamide (0·4)	100	1·2	4·0	5·0
Arylamidase	2	L-leucyl-β-naphthylamide HCl (0·4)	100	—	—	7·8

Stop-time assay was initiated by the addition of 0·1 ml epidermal extract (or 0·1 ml of 1 mg/ml BSA solution) to 0·1 ml buffered substrate (Table 2). After incubation for 30 min at 37°C, the reaction was stopped by the addition of 1·0 ml of carbonate/bicarbonate buffer (pH 10·5) and the fluorescence measured. The standard consisted of 0·2 ml of NA (10^{-5} mol/l) added to 1·0 ml carbonate/bicarbonate buffer. After substraction of the blank, enzyme activity was calculated as before.

Cathepsins D and E. These were assayed by the release of fluorescein from protein–fluorescein conjugates according to de Lumen & Tappel (1970). Details of reaction conditions are given in Table 3.

The reaction was initiated by the addition of 0·1 ml epidermal extract (or 0·1 ml of 1 mg/ml BSA solution) to 0·1 ml buffered substrate. A standard (0·2 ml of 10^{-5} mol/l fluorescein) was included in each batch. After incubation for 3 h at 37°C, the reaction was stopped by the addition of 0·2 ml 'carrier' protein (20 mg/ml haemoglobin or albumin) followed by 0·6 ml trichloroacetic acid (5% w/v). The mixture was centrifuged, and 0·5 ml of the supernatant made alkaline with 0·5 ml of 0·4 mol/l NaOH. Fluorescence was measured ($\lambda_{ex} = 488, \lambda_{em} = 518$ nm); after subtraction of the blank, enzyme activity was calculated as nmol (10^{-9} mol) of acid-soluble fluorescein released per min per g epidermis.

TABLE 3. Assay systems for cathepsins D and E. Reaction was initiated by the addition of 0·1 ml epidermal extract to 0·1 ml buffered substrate. The activity of extracts was also tested against albumin–fluorescein and histone–fluorescein conjugates at pH 3·4

EC number	Enzyme	Concentration of epidermal extract (mg/ml)	Composition of buffered substrate			
			Substrate (mg/ml)	Phosphate (mmol/l)	Citrate (mmol/l)	pH
3.4.4.23	Cathepsin D	20	Haemoglobin–fluorescein conjugate (2·0)	50	50	3·4
3.4.4.–	Cathepsin E	20	Albumin–fluorescein conjugate (10·0)	50	50	2·5*

* Adjusted with HCl.

Effect of reaction variables

Experiments to determine the influence of pH, the effect of epidermal concentration and the effect of substrate concentration were conducted in the manner described previously (Mier & van den Hurk, 1975). In addition, studies were carried out to determine the effect of thiol reagents (-SH groups) and of chloride ions upon the activities of cathepsins B_1 and C. In all cases the precise reaction conditions (other than the variable being investigated) were as shown in Tables 1 and 3.

Progress curves were conducted at 37°C using the stop-time assay (Tables 2 and 3) for all enzymes.

Normal values

The activity of each enzyme was determined in fresh epidermal extracts prepared from 4 different guinea-pigs. Stop-time assays were used.

RESULTS

General

Four of the five substrates were hydrolysed at significant rate under the conditions described. The

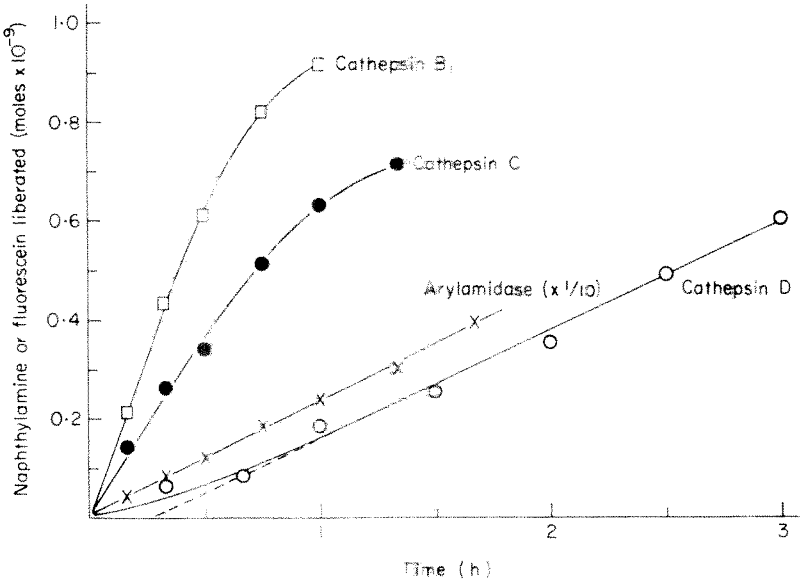

FIGURE 1. Progress curves showing the release of NA or fluorescein at periods up to 3 h. Measurements were made using the stop-time assay at 37°C.

remaining substrate (albumin–fluorescein conjugate) was hydrolysed at less than 5% of the rate of the corresponding haemoglobin–fluorescein conjugate. It was therefore assumed that cathepsin E was not present at measurable levels in guinea-pig epidermis; this enzyme was not investigated further.

Progress curves

These are illustrated in Fig. 1. It is seen that cathepsins B$_1$ and C are rather labile, the activity in

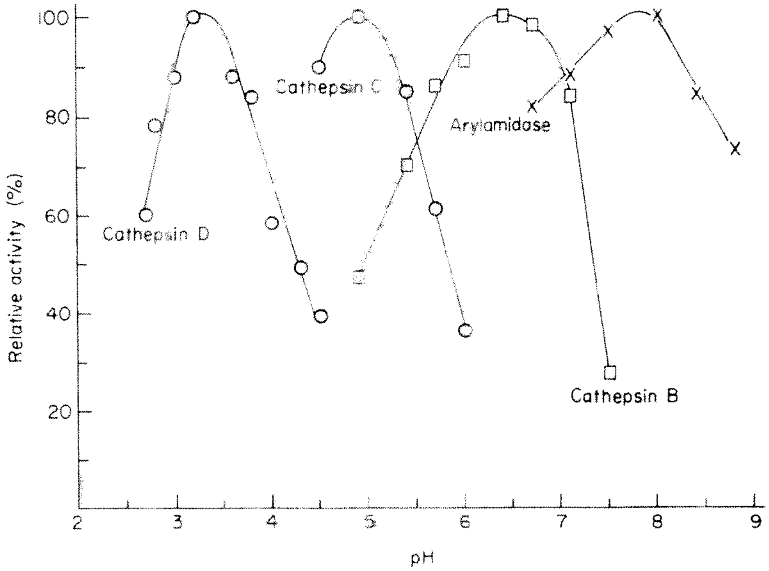

FIGURE 2. The effects of pH on the enzymatic activities.

TABLE 4. Properties of the peptide hydrolases of guinea-pig epidermis

Enzyme	pH optimum	K_m for substrate	Relative activity in the absence of:	
			Thiol reagents	Chloride ions
Cathepsin B_1	6·4	$6{\cdot}7 \times 10^{-3}$ mol/l	<20%	100%
Cathepsin C	5·0	$3{\cdot}6 \times 10^{-4}$ mol/l	<20%	negligible (K_m for chloride about $4{\cdot}7 \times 10^{-4}$ mol/l)
Cathepsin D	3·4	1·6 mg/ml	—	—
Arylamidase	7·8	$5{\cdot}7 \times 10^{-5}$ mol/l	—	—

both cases declining rapidly after about 30 min at 37°C. Cathepsin D and arylamidase, on the other hand, both appear to be relatively stable, the activities remaining linear for at least 3 h and 100 min, respectively.

Effect of epidermal concentration

The reaction rate was proportional to the concentration of epidermis in the added extract up to values of about 5, 2, 20 and 2 mg/ml for cathepsin B_1, cathepsin C, cathepsin D and arylamidase respectively.

Effect of pH

The variation of activity of each enzyme with pH is shown in Fig. 2, and the optimum values are listed in Table 4.

Enzyme kinetics

Lineweaver and Burk plots are illustrated in Fig. 3, and the Michaelis–Menton constants (K_m values) derived from these plots are listed in Table 4. It is seen that the plots of cathepsin C activity against GPNA (Fig. 3b) and of cathepsin D activity against haemoglobin–fluorescein conjugate (Fig. 3c) are both non-linear, suggesting substrate inhibition above concentrations of about 0·2 mmol/l and 1 mg/ml respectively.

Activator studies

Both epidermal cathepsin B_1 and cathepsin C were found to require thiol groups for full activity, the reaction rates being less than 20% of maximal in the absence of cysteine or cysteamine.

Epidermal cathepsin C appeared to have an absolute requirement for chloride ions, negligible activity being seen in its absence. The Lineweaver–Burk plot for chloride is shown in Fig. 4; extrapolation yields a K_m value of $4{\cdot}6 \times 10^{-4}$ mol/l. Cathepsin B_1 was unaffected by chloride ions up to a concentration of 20 mmol/l.

Normal values

The activity of each enzyme in fresh epidermal extracts prepared from four different animals is listed in Table 5. It is seen that both cathepsin D and arylamidase showed an unusually wide range; their ratio, however, remained roughly constant, suggesting that the observed variation was not the result of an experimental artefact.

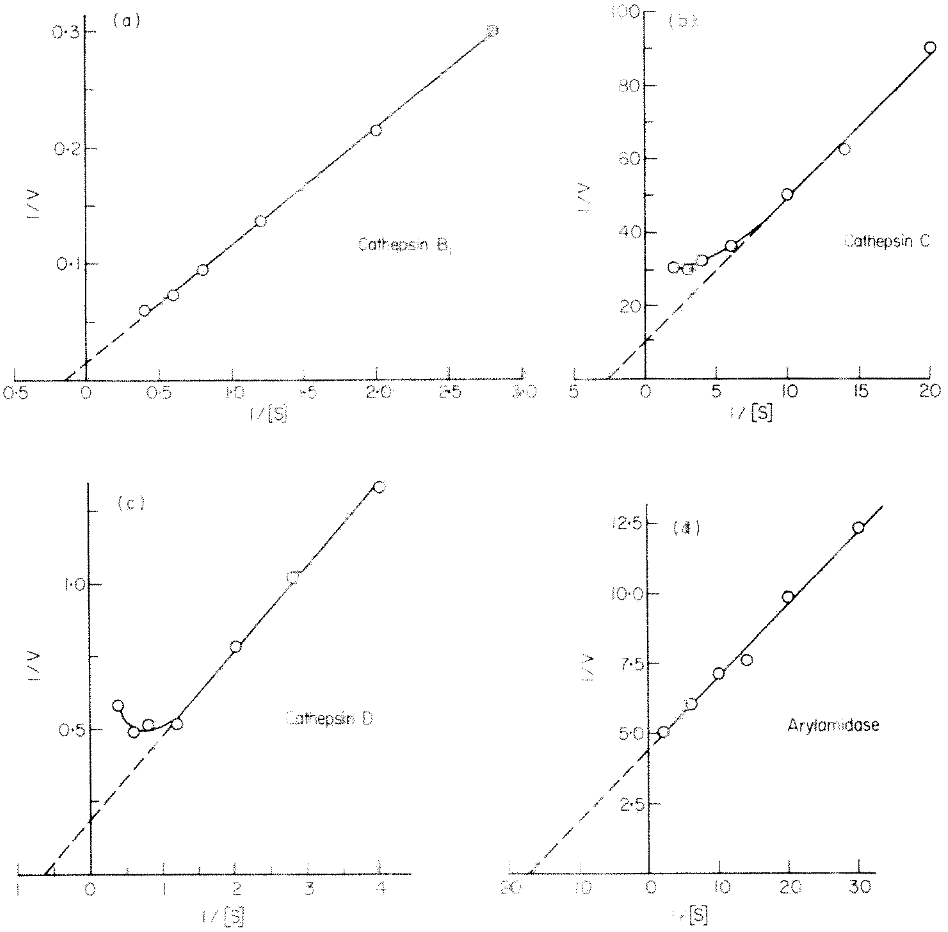

FIGURE 3. Lineweaver–Burk plots for substrates. Units are as follows: (a) V = nmol/min/g, S = mmol/l; (b) V = μmol/min/g, S = mmol/l; (c) V = nmol/min/g, S = mg/ml; (d) V = μmol min/g, S = mmol/l.

TABLE 5. Activities of epidermal peptidases in preparations from four different guinea-pigs. All values are in nmol (10^{-9} mol) of NA or fluorescein liberated per min per g fresh weight of epidermis

Enzyme	Animal no.				Mean
	1	2	3	4	
Cathepsin B_1	20	19	14	15	17
Cathepsin C	57	55	28	33	43
Cathepsin D	1·7	0·7	0·3	0·7	0·9
Arylamidase	390	75	50	84	150

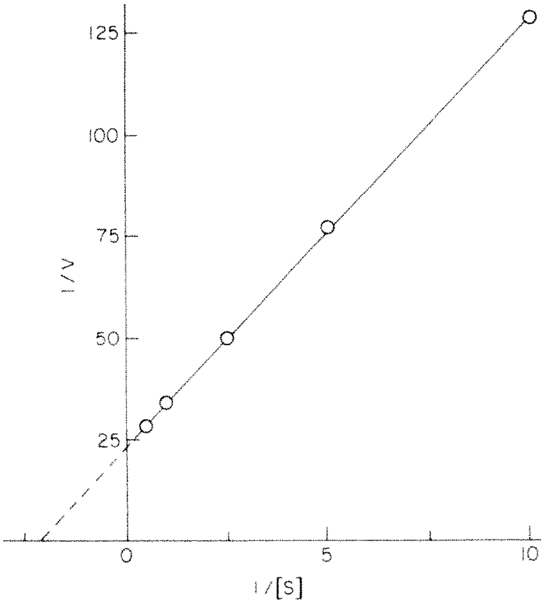

FIGURE 4. Lineweaver–Burk plot of epidermal cathepsin C for chloride. V = μmol/min/g and S = mmol/l.

DISCUSSION

A great deal of confusion in the earlier literature resulted from the overlapping substrate specificities of the peptide hydrolases. It is mandatory, when using crude tissue extracts, to define individual enzymes in terms of parameters such as pH optimum, Michaelis–Menton constant and activator requirements, in addition to specifying the nature of the substrate employed. In the following discussion, therefore, mention of specific reports will be made only when this data has been provided.

Cathepsin B_1

Lysosomal cathepsin B_1 is defined in terms of its relatively limited substrate specificity (amides or esters of benzoylarginine are usually employed), its pH optimum in the range 5–6·5, and its thiol (but not chloride) dependence. Our data (Table 4) confirm this enzyme to be present in guinea-pig epidermis.

Jansén & Hopsu-Havu (1969) investigated the ability of skin extracts to hydrolyse benzoylarginine NA; the activity (30 nmol/min/g fresh weight) and the pH optimum (6·0) which they reported for whole guinea-pig skin correspond closely with our values for epidermis. These authors found a similar pH optimum for the hydrolysis of casein, and commented that 'the same proteolytic enzyme' may have acted on both substrates. Since the endopeptidase activity of cathepsin B_1 is now well documented, there seems little doubt that this conjecture was correct.

Cathepsin C

Despite the much wider range of synthetic substrates attacked by this enzyme, lysosomal cathepsin C may be defined by its pH optimum (5–6) in conjunction with its thiol dependence and its absolute requirement for chloride. The data in Table 4 provide adequate confirmation of the presence of this enzyme in guinea-pig epidermis. It should be noted that, because of the low K_m for chloride, the requirement for this ion can be demonstrated only when using very dilute (or dialysed) epidermal

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extracts. In certain previous reports it is clear that possible chloride-dependence was obscured by the endogenous contribution of the tissue.

The first convincing description of cutaneous cathepsin C was apparently that of Hopsu-Havu & Jansén (1969b). These authors extracted and partially purified a thiol- and chloride-dependent enzyme active against alanylalanyl NA, which had a pH optimum of 5·0; curiously, it was present in whole human skin but not in rat skin. Subsequently de Bersaques (1970) quantitated cathepsin C in human epidermis and dermis. Using glycine-phenylalanine NA as substrate (pH 4·0), he obtained activities of 0·26 and 0·17 μmol/min/g dry weight respectively; these are quite similar to our value for guinea-pig epidermis.

Cathepsin D

This lysosomal endopeptidase is defined by its pH optimum (3–4), its lack of stimulation by thiol or chloride, and its marked preference for haemoglobin over other substrates tested. The epidermal activity described here fulfils these conditions; no measurable activity was found against either albumin–fluorescein or histone–fluorescein conjugates at pH 3·4. Our value of 1·6 mg/ml for the K_m of epidermal cathepsin D is almost identical to the figure of 1·5 mg/ml reported by de Lumen & Tappel (1970) for isolated liver lysosomes.

Lazarus & Dingle (1974) have recently demonstrated the presence of cathepsin D in whole rabbit skin. Their pH optimum of 3·2 is very close to that which we observed for the epidermal guinea-pig enzyme.

Arylamidase (amino acid naphthylamidase)

These terms cover the rather complex and still ill-defined group of enzymes which cleave amino acid naphthylamides. Leucine naphthylamidase has been shown to be lysosomal in origin, despite its neutral pH optimum (reviewed by Barrett, 1972).

Many workers have studied the hydrolysis of amino acid naphthylamides by skin extracts, perhaps the most extensive investigation being that of de Bersaques (1968). Using leucine NA as substrate, both the activity (6·3 μmol/min/g dry weight) and the K_m ($3·5 \times 10^{-4}$ mol/l) for human epidermis were an order of magnitude higher than our present values for guinea-pig epidermis. Panveliwalla & Moss (1966), on the other hand, obtained K_m values identical to our own for extracts of human kidney, intestine, pancreas and liver. It is clear that further work is needed before the questions of tissue and species variability of these enzymes can be answered with certainty.

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British Journal of Dermatology (1975) **93**, 519.

Livedo reticularis*

SIGNS IN THE SKIN OF DISTURBANCE OF BLOOD VISCOSITY AND OF BLOOD FLOW

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Accepted for publication 7 February 1975

SUMMARY

The clinical sign of livedo reticularis derives from stasis of blood in the superficial venous drainage systems of the skin. Many factors can delay the flow of de-oxygenated blood away from the skin, notably hyperviscosity of the blood itself and obstruction due to disease affecting dermal arteries, capillaries, or venules.

The pattern of health

The network (reticulate) pattern seen on the skin of healthy children as a physiological commonplace when they are chilled, cutis marmorata, is the image of the venous drainage system. When adults blush the meshes reassert themselves at certain sites, maybe even on the thighs. When the interlacing margins of the net have a bluish hue (livedo), this can be attributed to and explained by:

(1) *The anatomical disposition of the cutaneous blood vessels* and the flow routes of the blood through them. (Fig. 1). The diameter of the mesh of the venous network differs according to its anatomical situation—and is most easily seen in disease (Figs 2 & 3), and

(2) *The rheological properties of whole blood.* The increased viscosity at low temperatures reduces its rate of flow allowing more time for de-oxygenation of the blood, which, being in superficial vessels, appears livid within the skin.

The most important feature is the network pattern. In clinical practice the word livedo is so loosely interpreted that curiosities such as 'livedoid vasculitis' (Winkelmann *et al.*, 1974) creep into the argot, but this allows a wider range of reticulate vascular conditions to be discussed.

Renaut (1883), Unna (1896) and Adamson (1912), and later Spalteholz (1927), postulated that the round pink zones of skin, the interstices of the net, represent the bases of central cones supplied from their apices with fast flowing arterial oxygenated blood. The blood in each surface zone drains peri-

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* Read at the British Association of Dermatology Annual Meeting, July 1974.

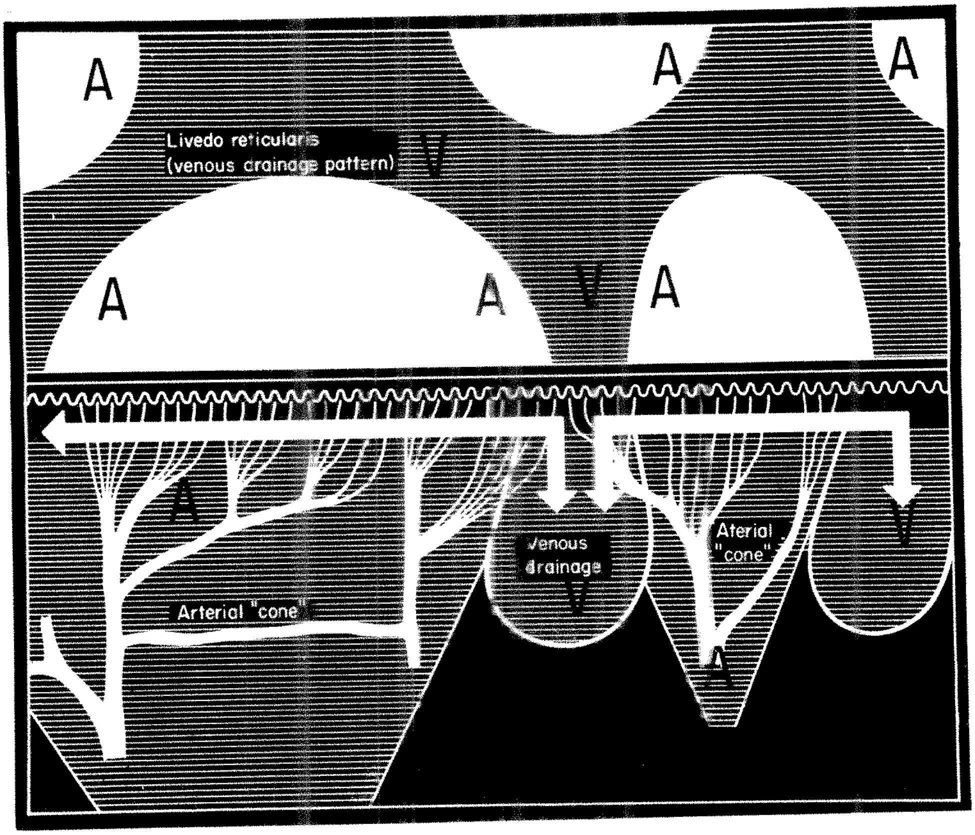

FIGURE 1. Upper part: Surface view of skin. (A) represents pink arterial zones supplied by the arterial cone: the livid network (V) comprises the contiguous venous drainage zones.
Lower part: Overall flow direction of upper dermal blood vessels is indicated by arrows. The blood from the arterioles supplies the 'capillary candelabra' which drains into the subpapillary venous plexus. These venules throughout the area drain peripherally and are close to the epidermis where they are vulnerable to the influence of cold which will alter the viscosity and flow rate of the whole blood within them.

pherally into a marginal network system of collecting venules along drainage channels splayed out horizontally close beneath the epidermis where they are vulnerable to the influence of surface temperature. Whole blood flows more sluggishly when chilled; its transit time is prolonged and more oxygen is lost. Being so superficial the blood is visible through the skin surface and the edges of each zone join to assume a reticulate pattern, livid due to the de-oxygenated haemoglobin.

The venous network that marginates the arterial zones is not to be thought of in conventional anatomical terms. It comprises an overall system and does not take into account the minor and highly organized sub-systems at different levels in the skin, such as those subserving the hair follicles and the sweat glands (Winkelmann *et al.*, 1961; Saunders, 1961). My diagram (Fig. 1) has been greatly simplified. The subpapillary venous drainage plexus in reality is a mass of interconnecting vessels anastomosing as a microscopically fine meshwork not only laterally parallel to the skin surface but also between several superficial and a number of deeper layers. Skin in the region of the microvessels subjected to years of venous stasis will suffer permanent anatomical distortion. The most superficial layer of papillary capillaries will have disappeared along with their supporting connective

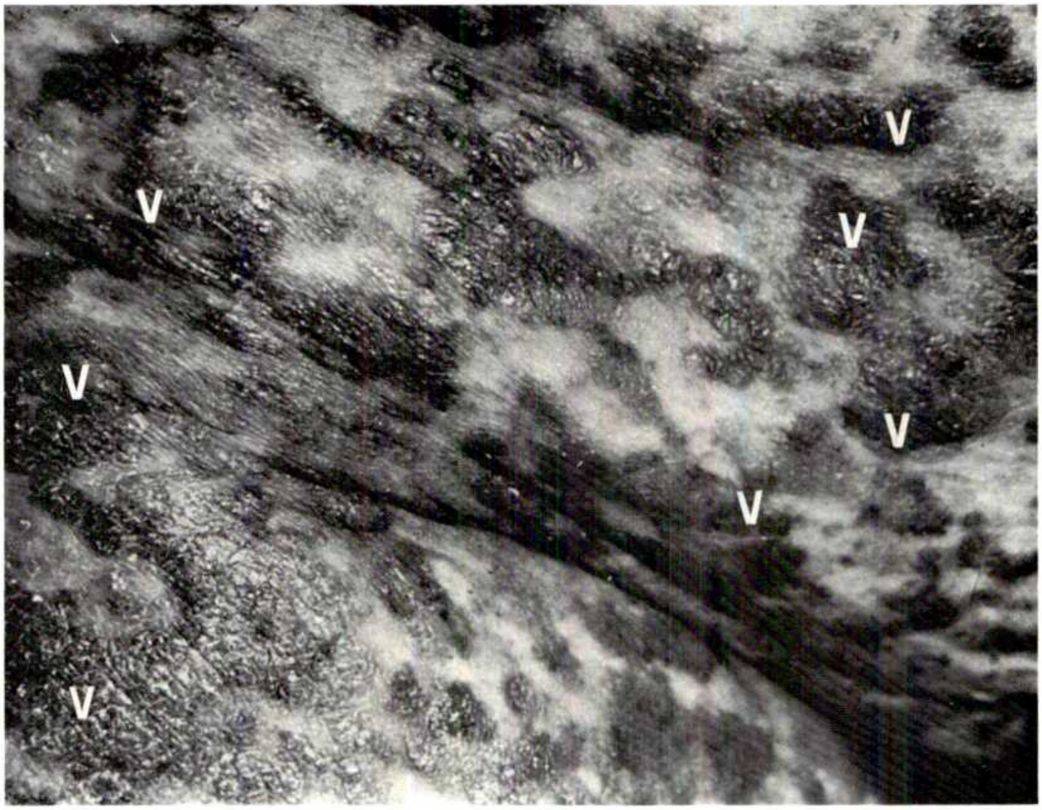

FIGURE 2. Disease is clinically apparent predominantly on the skin over the venous drainage system networks (V) rather than in the zones supplied by the arterioles. The mesh varies in diameter according to the anatomical site of the disease.

tissue. The horizontally disposed venules of the layers slightly deeper to these become dilated. These vessels thereby are now closer to the epidermis and the blueness of the yet slower flowing blood within them is more obvious to the observer, and can be verified by capillary microscopy (Champion, 1965; Ryan & Copeman, 1969). When the venules are inflamed as in cutaneous angiitis (Fig. 4), the net and its interstices may be easily appreciated.

External thermal injury to healthy skin

The main damage to healthy skin inflicted by external temperature extremes is in the venular drainage areas because it is there that heat and cold linger, whereas the fast flowing arterial blood flushes the arterial zones and ensures swifter temperature equilibration. Cold (chilblains) or heat (erythema ab igne) induced disease may cause the blood to clot within the injured vessels and thereafter the adjacent tissues may never recover. This may be observed directly in the microvessels by capillary microscopy on chilling the posterior nailfolds of patients with connective tissue disorders (Copeman, 1968; Ryan & Copeman, 1969), or in the development of micropapular chilblains (Copeman, 1973). Or the injury will be seen clinically over the broad areas of the venous network as signs of inflammation,

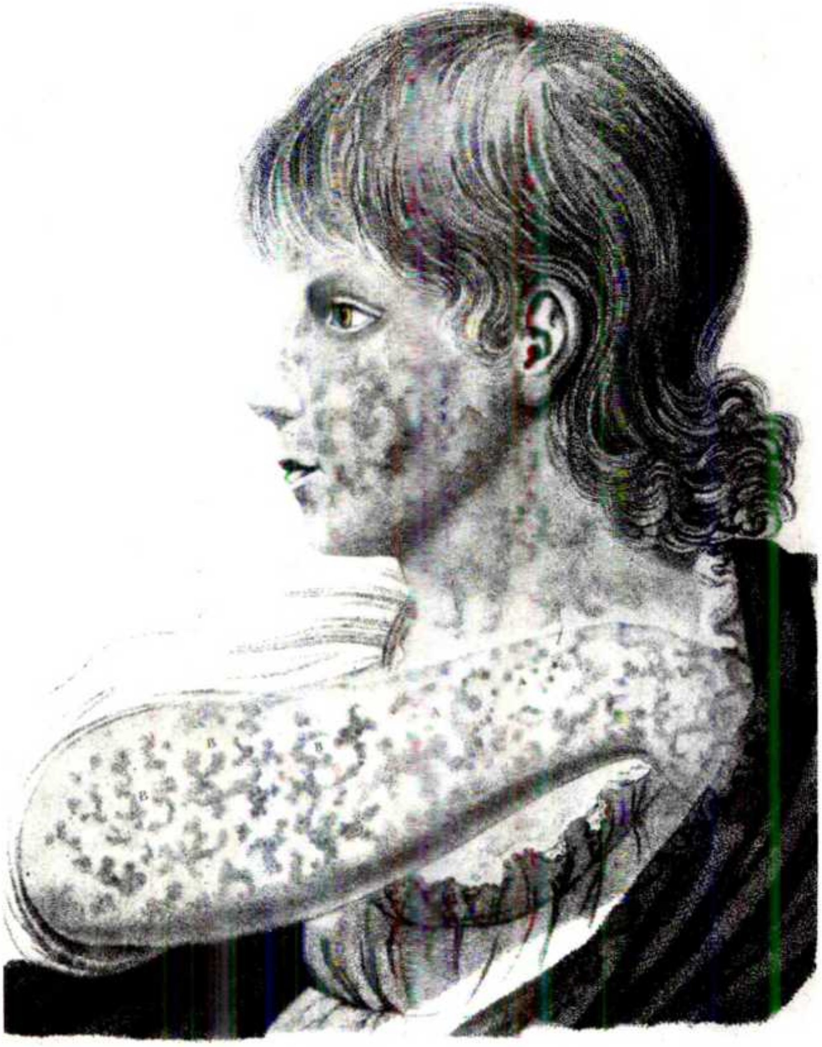

FIGURE 3. Toxic erythema. Efflorescence arranged in a reticulate venous pattern. Taken from coloured lithograph of Robert Willan (1798–1808), plate 21 in Order III 'Exanthemata or Rashes' entitled 'measles or rubeola sine catarrho.' Willan in his volumes discussed and illustrated the vascular basis of several other eruptions.

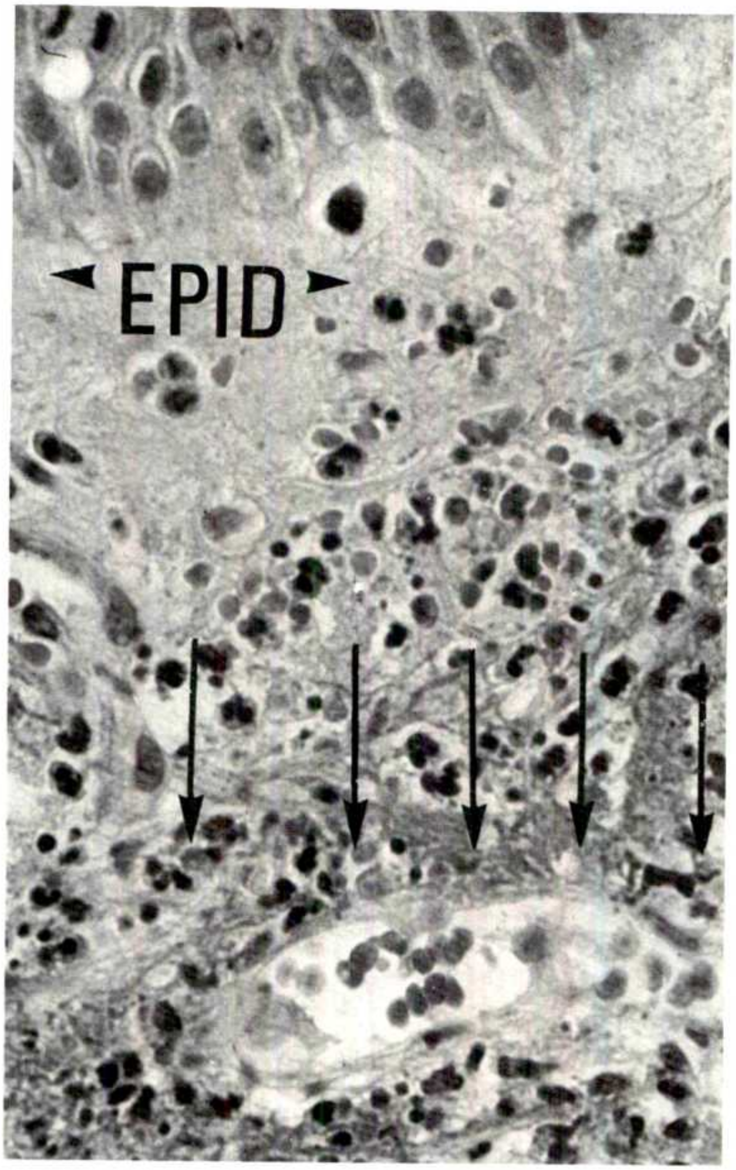

FIGURE 4. Cutaneous venulitis. Dilated horizontally disposed sub-papillary venous plexus (↓), splayed out parallel to the skin surface (EPID).

blisters ('vesicating fire-stains' of Hutchinson, 1898), ulcers, scars and post-inflammatory hyperpigmentation.

A reticulate livedo may be seen in certain neurological disorders: Parkinson's disease; strokes (Sneddon, 1965) where one may not be able to determine whether the signs in the skin derive from a primary thrombotic disease that also caused the cerebrovascular incident or are secondary to local or general vascular change associated with the ictus; paralysis as in poliomyelitis (Champion, 1965); and disturbances of the autonomic system. In these instances it is questionable sometimes whether the skin itself remains entirely healthy; the dermis may atrophy so that the vessels are more visible. Inactivity of the limb muscles and thus a poor venous blood return flow whether due to paralysis, or an orthopaedic splint, will exaggerate the signs (Copeman, 1972). The explanation may lie also in the susceptibility to chilling, lack of movement lessening the generation of body heat from the muscles. In an angiitis, the 'muscle pump' may fail to speed venous blood return so that the rash in the paralysed limb may be more profuse and may be seen particularly in the venous network areas. The exanthems of the common virus fevers of childhood (Fig. 3) and curiously the lesions of psoriasis and lichen planus (Fig. 5), the Köbner or isomorphic phenomenon, may be seen to be clustered in the pattern of this venous net.

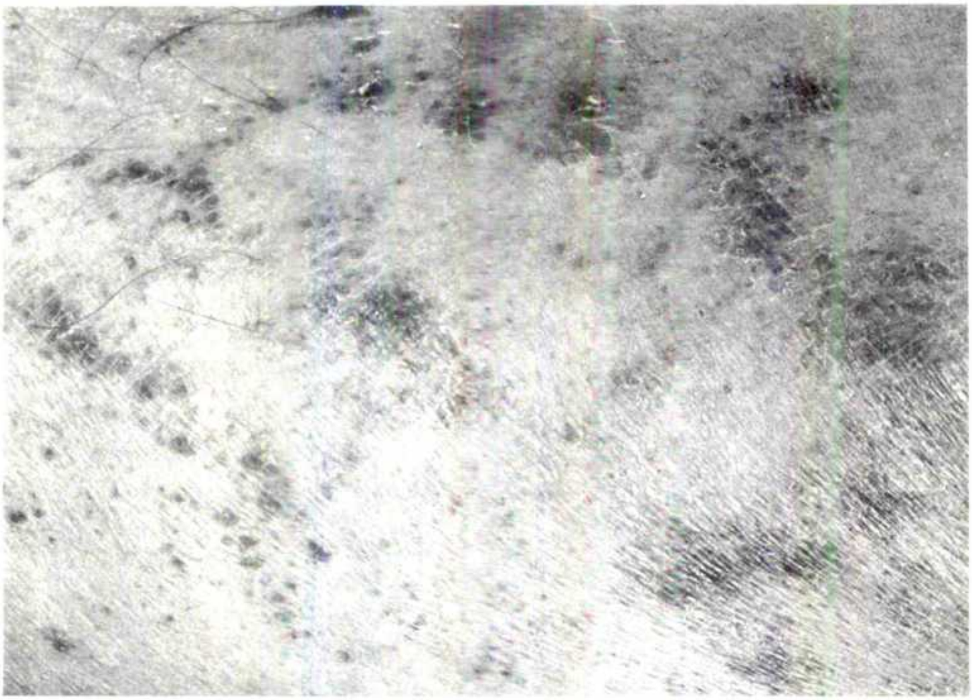

FIGURE 5. Non-vascular lesions clustered in reticulate pattern. Lichen planus.

'Yet, I applaud the Fashion's new pursuit
Of maxi, midi, and the trouser suit
Saving long legs from *Acrocyanosis*,
Chilblains upon the thigh, and *Vein thrombosis*;
While find I comfort hearing from the wise
'When Hem-Lines fall, Stock-Markets then do rise'
Anon

Livedo reticularis: sign of internal disease

Livedo reticularis is a clinical sign that can result from a number of different pathological sequences (Champion, 1965, 1972) just as may occur in other common skin conditions where one set of signs may be the end result of numerous disease processes or aetiological agents; the venular disorder of atrophie blanche (Copeman & Ryan, 1971) is another example. Livedo reticularis may prove therefore to be a valuable presenting sign harbinging grave systemic disease, or an adjuvant to a clinical diagnosis suggesting lines of further laboratory investigations. Erasmus Wilson (1855) was aware of its systemic significance when he noted 'melanopathia (syphilitica)' to take up a 'definite pattern like stains on the skin of a corpse or dark hair on a dappled grey horse'. Adamson (1912) was more precise and stated that the spirochaete settles where there is stasis in the venous network area of reduced circulation and the signs become more marked on exposure to cold. I shall explain the physical sign on the basis of the anatomical site of the pathology in the dermal blood vessels. Similar clinical signs result whichever of the three segments of the vasculature is primarily diseased (Fig. 6).

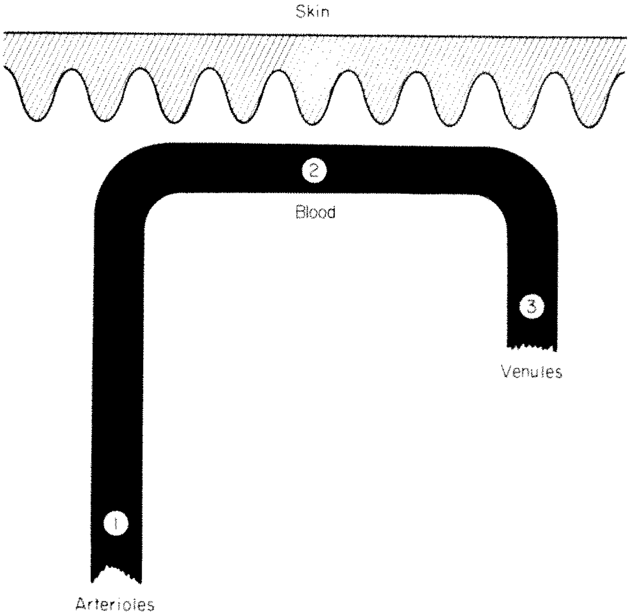

FIGURE 6. Livedo reticularis. 3 origins.

(1) *Arterioles:* obstruction to the inflow of blood.

(2) *Capillaries:* hyperviscosity of the blood flowing within them with or without primary physical damage to the microvessels.

(3) *Collecting venules*: obstruction to the outflow of blood.

The conditions underlying these disorders include those listed in Table 1. The classification is by no means precise and some of the diseases due to inflow obstruction for example could have been listed under the other headings. Systemic lupus erythematosus is historically an arterial disease. Foremost today is its cardinal characteristic of prodigal antibody formation and being cryoprecipitable or of a large size the antibody may cause blood hyperviscosity. We recognize now that the main vascular brunt probably falls on the capillaries and venules where immunological responses damage their walls and clog the blood flow. (Medical opinion continues unthinkingly to

TABLE 1. Livedo reticularis (systemic disease)

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(1) *In-flow obstruction*

Arteriosclerosis; multiple small emboli; polyarteritis nodosa; temporal (giant cell) arteritis; systemic lupus erythematosus; 'arteritis' of rheumatoid disease; hyperparathyroidism and hypercalcaemia.

(2) *Hyperviscosity of blood and blood stasis in capillary-venules*

- (i) Cells: polycythaemia, thrombocythaemia.
- (ii) Plasma: cold precipitable proteinaemias of all types (cold agglutinins, cryofibrinogens, cryoglobulins, etc); macroglobulinaemias.
- (iii) Ill defined hypercoagulable states (decreased fibrinolysis or increased fibrin deposition).
  - (a) Locally in blood vessels.
  - (b) Generally in blood.
- (iv) Neurological disease or injury.
- (v) Drugs acting on blood vessels directly (physiological, toxic, immunological) or via the autonomic nervous system. Liberation of natural vasoactive pharmacological agents (carcinoid syndrome).
- (vi) Primary pathology in vessels, e.g. cutis marmorata telangiectasia congenita.

(3) *Out-flow obstruction*

- (i) Immune and protein complex deposition ('drug rashes', 'connective tissue' disorders, etc.). Cryoglobulinaemic venulitis. Cutaneous venulitis (angiitis), 'livedoid' vasculitis, erythema elevatum diutinum.
- (ii) Endotoxin damage (generalized or local Shwartzman reactions). Enzyme damage, e.g. acute pancreatitis.
- (iii) Infections (syphilis; tuberculosis; meningococcaemia, streptococcaemia; rickettsia; viruses)—immune mechanisms often operating also.
- (iv) Deposition of crystals: hypercalcaemia, hyperoxaluria.

Out-flow obstruction will be accentuated by haemodynamic back pressure as in heart failure and dependency. The several causes of 'venous gangrene'. Many of the conditions are self-perpetuating. Infections: micro-organisms may act directly upon the vascular endothelium or indirectly through allergic mechanisms or occasionally in both manners.

(4) *Heat exchange defects*

Thermal damage (chilblains, and radiant heat injury).

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associate livedo reticularis with lupus erythematosus and thus erroneously the two diseases are linked to arterial disease.)

Making the diagnosis of livedo reticularis does not absolve one from determining the pathogenesis of this clinical pattern in the patient. A drug reaction may reveal itself as direct toxic injury to the vessels or through immune mechanisms. Primary hyperparathyroidism and hypercalcaemic states can cause calcification of the deep dermal muscular vessels as may chronic renal disease (Winkelmann & Keating, 1970). After renal transplantation or dialysis the signs of livedo reticularis may resolve. Calcium oxalate crystal deposition in the venular network pattern may be due to one of the hyperoxaluric states (Jansen, Groeneveld & van der Meer, 1974).

To date, many of the hypercoagulable states apart from the frank hyperviscosity syndromes and disseminated intravascular coagulopathies are ill defined. Their investigation requires research procedures on the blood and special histopathological examination of the blood vessels that are far from feasible standard practice. The situation within the skin microvessels where complex inter-relationships of blood elements and blood flow factors occur (Copeman & Ryan, 1971) usually has to be deduced by sampling blood from the general circulation. A gross defect such as a vastly elevated level of fibrinogen in venous blood of a patient might have been the cause of the coalescing network of ischaemic ulcers on the legs (Tan & Copeman, 1974), although the precise details of the balance between coagulation and fibrinolysis within the affected cutaneous microvessels were not assessable. Similar signs can be seen in paraproteinaemias as in diseases such as lichen myxoedematosus. In cutis marmorata telangiectasia congenita (Way *et al.*, 1974) the defect is probably primarily in the capillaries and venules, and the consequence of this is intravascular stasis and thrombosis which cause the ulcers.

Further development of specialized histopathological studies of the cutaneous blood vessels of the type developed by Ryan (1973) might detect a local defect of coagulation—fibrinolysis balance in those patients with the curious and rare variety of 'idiopathic' livedo reticularis that relentlessly extends over limbs and trunk from early adult life. Some of the rare cutaneous vascular effects seen in neoplastic disease may be explainable on the basis not of allergic phenomena but of intravascular coagulation defects (Owen & Bowie, 1974), alone.

Certain drugs with perhaps an unsuspected vaso-activity can be responsible for the pattern, notably amantadine (Vollum, Parkes & Doyl, 1971), which may deplete the catecholamine stores in relation to the sympathetic nervous system and allow venular and capillary vasodilatation and even intravascular clotting. Ignorance as to the pathogenesis of acrocyanosis (Champion, 1965; Copeman, 1973) makes it difficult to place confidently in the Table. The lesions of cutaneous angiitis (venulitis) are often distributed in a reticulate manner. This condition, (encumbered by so many ponderous terminological oddities (Copeman & Ryan, 1970), now hopefully garnered under this one title (Copeman, 1975)), has many aetiologies. The pattern may be seen in rashes presumed to be drug induced, various infections particularly when these are being treated by antibiotics, connective tissue disorders, and internal neoplasms. In those patients in whom the pathogenesis can be unravelled, clinical and histopathological factors point towards immune complex deposition in the upper dermal venules (Copeman, 1970). The primary allergic response damages the venules and causes, as a secondary non-specific effect, blood stasis, clotting (Copeman & Ryan, 1971), rerouting of blood, and other flow disturbances of the microcirculation of the superficial dermal plexuses; perhaps abetted by such physical factors as dependency and chilling (Ryan & Copeman, 1969; Copeman, 1972). Nonetheless this network pattern is seen in diseases in which the vascular relationship appears to have no obvious direct connection with pathogenesis. Some of the conditions are dermatological curiosities but others have sinister systemic significance relating to development at a young age of neoplasms or immunodeficiency defects, hazardous to the patient. I consider that they are best listed separately to avoid confusion until such time as an hypothesis to explain the distribution of their clinical signs is advanced (Table 2).

CONCLUSIONS

The clinical sign, livedo reticularis, can be explained as a pathological progression of: (1) the natural

TABLE 2. Examples of diseases where the signs on the skin may be arranged in a reticulate pattern but the precise pathogenetic relationship with blood vessel disease is ill understood or of seemingly secondary importance

Blood vessels	Congenital vascular malformations and developmental defects. Capillary haemangiomas. Giant glomangioma. Angioma serpiginosum. Urticaria pigmentosa.
Genodermatoses	Hereditary telangiectatic syndromes (Rothmund-Thomson, Bloom, ataxia telangiectasia, and others). Reticulate pigmented anomaly of the flexures (Dowling, Degos). Incontinentia pigmenti.
Cutaneous reticuloses and 'the poikilodermas'	Poikiloderma atrophicans vasculare, poikilodermatomyositis.
Others	Pityriasis rubra pilaris. Nekam's porokeratosis striata.

viscosity properties of whole blood and (2) stasis of blood in the delicate tracery of the filigree plexuses of the subpapillary venous drainage systems. These underlie a network of contiguous areas in the skin, the frame of the net being of different size according to its anatomical site.

Various forerunners may be responsible for the blood disorder that results in hyperviscosity, blood stasis, and clotting.

Any factor that delays the flow of blood out through the venular channels will predispose to the physical signs. The extra de-oxygenation of the blood due to its lingering in the drainage network and prolongation of its transit time will accentuate the contrast of the livid blood with that of the interstices, the central pink zones supplied by the arterioles.

The blood vessels, arterioles, capillary microvessels, or the collecting venules, may each be the principal site of diseases. Hitherto, in livedo reticularis, the arterial side of the vascular tree has been stressed but similar signs will result from obstruction of blood flow in the other skin blood vessels. The nature of the underlying pathological disturbance is relatively unimportant in determining the signs, and it may be a developmental defect, an angiitis, infective or immunological, a degenerative disorder, and suchlike.

ACKNOWLEDGMENT

The diagrams were made by the Department of Medical Illustration, Westminster Hospital.

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The significance of a positive cutaneous immunofluorescence test in systemic lupus erythematosus

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Accepted for publication 10 February 1975

SUMMARY

Direct cutaneous immunofluorescence microscopical examination of uninvolved skin is an important diagnostic test in systemic lupus erythematosus. Its prognostic significance is undetermined. In twenty-four patients there was an increased incidence of leukopenia, hypocomplementaemia, and LE cells in patients with positive skin immunofluorescence. Positive cutaneous immunofluorescence of uninvolved skin was correlated with the most severe forms of lupus renal disease, membranous glomerulonephritis, and diffuse proliferative glomerulonephritis.

Systemic lupus erythematosus (SLE) is a chronic multisystem disease. Laboratory abnormalities include LE cell phenomena, antinuclear antibodies (ANA), elevated erythrocyte sedimentation rate, leukopenia, abnormal urinalysis, rheumatoid factor, biological false positive serology (VDRL), and decreased serum complement levels (Tuffanelli, 1972). Numerous auto-antibodies, including those against DNA (Holman, 1960), RNA (Schur *et al.*, 1971), leukocytes, lymphocytes (Terasaki *et al.*, 1970), erythrocytes, platelets, histone, nucleoprotein, extractable nuclear antigen, ribosomes (Sturgill & Preble, 1967), and lysosomes (Bell *et al.*, 1971) have also been described. In 1963, Burnham *et al.* reported immunoglobulins and complement at the dermo–epidermal junction in patients with lupus erythematosus. Numerous investigators have confirmed this and homogeneous, granular and thready patterns of immunofluorescence have been described (Cormane, 1964; Tan & Kunkel, 1966; Pohle & Tuffanelli, 1968; Percy & Smyth, 1969; Kay & Tuffanelli, 1969; Burnham & Fine, 1971). Direct immunofluorescence staining is found in approximately 90% of patients with systemic and discoid lupus erythematosus (DLE) when taken from involved skin but the test is positive in only 50–60% of patients with SLE when done on uninvolved skin and it is never positive in uninvolved skin of patients with DLE (Tuffanelli, 1972). 'False positive' reactions have been reported with rosacea, facial telangiectasia and dermatomyositis (Jablonska *et al.*, 1970).

The direct immunofluorescence examination has become an important diagnostic test in systemic lupus erythematosus but its prognostic and laboratory significance has not yet been determined.

Caperton *et al.*, (1972) have examined twenty-nine patients with systemic lupus erythematosus for cutaneous immunofluorescence and renal disease and concluded that no relationship exists. Burnham & Fine (1971) and Gilliam *et al.* (1974), however, stated that kidney disease is more common in patients with dermo–epidermal deposition of immunoglobulins and that positive cutaneous immunofluorescence is an indication for kidney biopsy.

We have examined twenty-four patients with systemic lupus erythematosus and have attempted to correlate cutaneous immunofluorescence with laboratory findings in these patients.

MATERIALS AND METHODS

Twenty-four patients with systemic lupus erythematosus were selected. Each patient had at least 4 of the 14 American Rheumatism Association criteria for the classification of systemic lupus erythematosus (Cohen *et al.*, 1971). There were nineteen women and five men aged from 16 to 55 years. Biopsy specimens were obtained from clinically normal skin of the lower back under local anaesthesia with 1 or 2% lidocaine. They were immediately frozen in either liquid nitrogen or dry ice and were sectioned in a cryostat dermatome to a thickness of approximately 4 μm. The specimens were stained with commercially available fluorescein-conjugated goat antihuman antisera to IgG, IgM, IgA, IgE and C3 and examined under a fluorescence microscope. The fluorescein/protein ratios, antibody concentrations and protein concentrations of the antisera used are listed in Table 1. Forty-one patients were selected as controls: dermatomyositis (3), scleroderma (5), atopic dermatitis (2), discoid lupus erythematosus (10), Sézary syndrome (1), lymphocytoma cutis (1), pyoderma gangrenosum (2), psoriasis (2), drug eruptions (2), pemphigus (2) and bullous pemphigoid (6). Six patients were on prednisone therapy at the time of biopsy (10–60 mg daily) and no patients were on immunosuppressive drugs. Blood tests (VDRL, LE cells, sedimentation rate, white blood count, rheumatoid factor, and serum complement levels) and urinalyses were performed at the Clinical Laboratory of the Albany Medical Center. Proteinuria was defined as greater than 500 mg per 24 h. Haematuria was defined as greater than 5 red cells per high power field. Kidney biopsies were performed by the Renal Division of the Department of Medicine and examined by the Pathology Department. All kidney biopsies were stained with haematoxylin and eosin and with periodic acid–Schiff stains and examined under the light microscope. Antinuclear antibodies were measured by immunofluorescence with freshly frozen rat liver using the technique described by Anderson *et al.* The antisera used for the ANA determination was commercially available goat antihuman globulin serum with a protein concentration of 8·0 mg/ml and a fluorescein/protein ratio of 8·3. All sera of patients were examined at dilutions of 1:10 and 1:50. All patients were followed for periods ranging from 3 months to 5 years and laboratory

TABLE 1. Fluorescein/protein ratios, antibody concentrations and protein concentrations of antisera used

	IgG	IgM	IgA	IgE	C3
Fluorescein/protein ratio*	4·0	2·6	2·5	2·2	3·4
Antibody concentration†	5·0	1·9	2·4	—	2·0
Protein concentration‡	20·0	20·0	21·9	11·1	12·3

*Measured as molar ratio; †Measured as mg/ml.; ‡Measured as mg/ml.

results reflect the most severe abnormalities during the evaluation period for each patient. The log-likelihood, χ^2 test was used for analysis of data.

RESULTS

The results of immunofluorescence are summarized in Table 2. 13/24 or 54% of the skin biopsies had immunoglobulins at the dermo-epidermal junction. All control patients had negative immunofluorescence, except for the patients with bullous pemphigoid who had IgG and C3 at the basement membrane and the three patients with pemphigus who had epidermal intercellular staining with IgG.

TABLE 2. Results of direct immunofluorescence on uninvolved skin

Patient	IgG	IgM	IgA	IgE	C3
1	+	+	—	—	—
2	+	+	—	—	—
3	—	—	—	—	—
4	—	—	—	—	—
5	—	—	—	—	—
6	—	—	—	—	—
7	+	+	—	—	—
8	+	+	—	—	+
9	+	+	—	—	—
10	+	—	—	—	—
11	+	+	—	—	—
12	+	+	—	—	—
13	—	—	—	—	—
14	+	+	—	—	—
15	—	—	—	—	—
16	—	+	—	—	—
17	+	+	—	—	+
18	—	—	—	—	—
19	—	—	—	—	—
20	—	+	—	—	+
21	—	—	—	—	—
22	+	—	—	—	+
23	—	—	—	—	—
24	—	—	—	—	—

The clinical and laboratory findings are summarized in Table 3. 12/24 or 50 % of the patients had cutaneous lesions; 22/24 or 95% had arthralgia or arthritis; antinuclear antibodies were present in 22/24 or 93% of the patients: 14/24 or 58% had a positive LE cell preparation; elevated erythrocyte sedimentaion rate was present in 23/24 or 96%; 20/24 or 83% of the patients had leukopenia; eleven patients had focal proliferative glomerulonephritis; two had diffuse proliferative glomerulonephritis; four had membranous glomerulonephritis; and five had normal kidney biopsies; rheumatoid factor was measurable in five patients and had three false positive VDRL.

TABLE 3(a). Laboratory findings in patients with systemic lupus erythematosus

Patient	Skin lesions	Arthritis/ arthralgia	LE cells	ESR	WBC	C3*
1	+	+	+	48	2900	31
2	—	+	+	31	3000	120
3	—	+	+	92	6090	105
4	+	+	—	96	4000	77
5	+	—	—	18	4200	150
6	+	+	+	97	2300	135
7	+	+	—	63	9700	185
8	+	+	—	30	5000	Not done
9	—	+	+	83	3400	Not done
10	—	+	—	98	4200	300
11	—	+	—	44	5400	98
12	+	—	—	152	4900	40
13	—	+	+	107	3700	260
14	—	+	+	68	3300	55
15	—	+	—	84	4800	94
16	+	+	+	107	1300	125
17	—	+	+	98	2300	107
18	+	+	+	46	3800	82
19	—	+	—	79	4400	120
20	+	+	—	28	4900	80
21	+	+	—	28	4400	162
22	+	+	—	94	2700	53
23	+	+	—	80	4000	125
24	—	+	—	60	4200	80

*C3, third component of complement; normal 97–189 mg/100 ml.

DISCUSSION

Our findings suggest that a correlation exists between the severity of renal disease and cutaneous immunofluorescence. 5/6 or 83% of the patients with membranous and diffuse proliferative glomerulonephritis, which are the most severe forms of lupus renal disease (Baldwin *et al.*, 1970), had positive cutaneous immunopathology while only 3/16 of the patients with focal proliferative glomerulonephritis or normal kidney biopsies had positive skin biopsies ($P<0{\cdot}05$). Conversely, 5/10 of the patients with positive immunofluorescence developed severe renal disease while only 1/12 with negative immunofluorescence had severe kidney disease ($P<0{\cdot}05$).

There was a correlation between the severity of leukopenia and cutaneous immunofluorescence. 7/8 of the patients with a white blood count less than 3500 cells/mm^3 had positive immunofluorescence. Conversely, 7/13 of the patients with positive immunofluorescence developed a WBC of less than 3500 cells/mm^3 while only one patient (1/11) with negative skin biopsies developed a leukopenia of less than 3500 cells/mm^3 ($P<0.05$).

No relationship was found between the presence and pattern of ANA and cutaneous immunofluorescence. 12/13 of the patients with positive cutaneous immunofluorescence had antinuclear antibodies while 10/11 of the patients with negative cutaneous immunopathology had antinuclear antibodies in the serum. LE cells, however, were more common in patients with dermo–epidermal immunoglobulins. 9/12 of the patients with positive skin biopsies had LE cells while 5/10 of the patients with negative cutaneous immunopathology developed LE cells.

TABLE 3(b)

Patient	Urinalysis	Kidney biopsy	RA†	VDRL	ANA
1	Proteinuria hyaline casts	MGN	—	—	+
2	Normal	Normal	—	—	+
3	Normal	FGP	+	—	+
4	Proteinuria	DPG	—	—	+
5	Normal	Normal	—	—	—
6	Proteinuria	Normal	+	—	+
7	Proteinuria	MGN	Not done	—	—
8	Normal	Not done	Not done	—	+
9	Proteinuria	DPG	—	—	+
10	Normal	Normal	—	—	+
11	Proteinuria	MGN	—	+	+
12	Normal	Normal	—	—	+
13	Normal	FPG	—	—	+
14	Proteinuria haematuria	FPG	—	—	+
15	Proteinuria granular casts	FPG	—	—	+
16	Proteinuria	MGN	+	+	+
17	Proteinuria granular casts	FPG	—	+	+
18	Normal	FPG	+	—	+
19	Proteinuria haematuria	Not done	—	—	+
20	Normal	FPG	—	—	+
21	Normal	FPG	—	—	+
22	Proteinuria granular casts	FPG	+	—	+
23	Normal	FPG	—	—	+
24	Normal	FPG	—	—	+

†RA, Rheumatoid factor. MGN, Membranous glomerulonephritis; FPG, Focal proliferative glomerulonephritis; DPG, Diffuse proliferative glomerulonephritis.

The erythrocyte sedimentation rate, which is often used as an indication of disease activity, had no relationship to skin biopsies. Serum complement levels, which have more recently been advocated as a measure of disease activity, correlated well with the cutaneous immunopathology. All patients with a complement (C3) level less than 60 mg/100 ml had positive immunofluorescence. However, no statistical difference was noted in patients with mild hypocomplementaemia (60–97) and those with normal complement levels ($P<0{\cdot}05$).

Rheumatoid factor, which is frequently positive in systemic lupus erythematosus and in relatives of patients with SLE (Tuffanelli, 1972), and VDRL, which is often falsely elevated in SLE, did not correlate with cutaneous immunofluorescence.

TABLE 3(c)

Patient	ANA pattern	ANA titre
1	Peripheral, homogeneous and speckled	1 : 50
2	Peripheral	1 : 50
3	Peripheral and speckled	1 : 50
4	Peripheral	1 : 50
5	Negative	1 : 10
6	Peripheral and reticulated	1 : 50
7	Negative	1 : 10
8	Peripheral and homogeneous	1 : 50
9	Peripheral, homogeneous and speckled	1 : 50
10	Homogeneous and reticulated	1 : 50
11	Speckled	1 : 50
12	Speckled	1 : 50
13	Homogeneous and speckled	1 : 50
14	Peripheral and speckled	1 : 50
15	Peripheral and speckled	1 : 50
16	Peripheral and speckled	1 : 50
17	Homogeneous	1 : 50
18	Peripheral, homogeneous and speckled	1 : 50
19	Peripheral and homogeneous	1 : 50
20	Speckled	1 : 50
21	Peripheral	1 : 50
22	Peripheral and homogeneous	1 : 50
23	Homogeneous	1 : 50
24	Homogeneous	1 : 50

CONCLUSIONS

The incidence of the most severe forms of lupus renal disease, membranous glomerulonephritis and diffuse proliferative glomerulonephritis, was increased in patients with positive cutaneous immunofluorescence on uninvolved skin. There was no relationship between cutaneous immunopathology and the presence and pattern of antinuclear antibodies, erythrocyte sedimentation rate, urinalyses, rheumatoid factor or biological false positive serologies. Positive cutaneous immunofluorescence is associated with a more severe form of lupus erythematosus. The direct cutaneous immunofluorescence test using uninvolved skin is an important prognostic test in systemic lupus erythematosus.

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Cellular immunity and circulating antibody to herpes simplex virus in subjects with recurrent herpes simplex lesions and controls as measured by the mixed leukocyte migration inhibition test and complement fixation

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Accepted for publication 13 February 1975

SUMMARY

Cellular immune responses and circulating antibody levels to herpes simplex virus were examined in patients with recurrent herpes simplex (HSV) infections and controls.

Mixed leukocyte migration inhibition by herpes simplex antigen was less in affected patients than in controls but serum antibody levels were higher.

There was no significant difference in leukocyte migration between patients with active or recent lesions and other herpes subjects, and the response in the mixed leukocyte migration test to phytohaemagglutinin (PHA) was similar in patients and controls.

The data presented suggest that a localized defect in cell mediated immunity to herpes simplex virus may exist and be responsible for recurrent herpes simplex infections.

Following primary infection with the herpes simplex virus, the virus establishes itself within the host, probably in or near nerve tissue and in the normal individual this provokes cellular and humoral immune responses which protect against re-infection and re-activation. However, in some subjects there is deficient protection and in these recurrent lesions occur (Juel-Jensen, 1973).

Previous studies of cellular immunity to HSV have been carried out using lymphocyte transformation, lymphocyte cytotoxicity, guinea-pig macrophage migration inhibition and skin testing but the results have been confusing (Wilton *et al.*, 1972; Russell, 1974).

In this study we have used both the mixed leukocyte migration inhibition test as a measure of delayed hypersensitivity and complement fixation as a measure of circulating antibodies. The former has been established as a measure of cellular immunity in many different systems and correlates with delayed skin reactions (Bloom *et al.*, 1973).

MATERIALS AND METHOD

For the leukocyte migration inhibition test a modification of the technique of Søborg & Bendixen (1967) was used.

20 ml blood samples were taken using Gillette syringes and divided between four tubes, each containing 0·3 ml of preservative-free heparin.

The white cell rich supernatant was collected, pooled and centrifuged at 200 ***g*** for 5 min. The cell pellet so obtained was washed three times in Hanks' balanced salt solution (Wellcome Reagents Ltd.) previously warmed to 37°C. The plasma was collected and stored at −40°C for antibody determination. Following washing, the cell pellet was re-suspended in TC199 (Wellcome Reagents Ltd.) containing 10% of un-inactivated horse serum. The volume used depended on the size of the cell pellet and was between 1 and 2 ml. Microhaematocrit capillary tubes 75 mm in length (Gelman & Hawksley) were filled with the suspension, sealed at one end with Cristaseal (Hawksley), packed into test tubes and centrifuged at 200 ***g*** for 10 min.

The capillaries were cut at the cell-fluid interface, the cell pellet being between 1 and 3 mm in length. The tubes were mounted singly in migration chambers (Sterilin) fixed with a small 'dab' of silicone grease, the open end of the tube pointing towards the centre of the chamber.

To each chamber (0·5 ml vol.) was added 0·05 ml of one of the following solutions, each in triplicate:

Normal saline,
Phytohaemagglutinin (PHA) 50 µg/ml saline,
HSV antigen: undiluted,
diluted 1/5
1/50
1/250 in saline,
HSV control antigen: undiluted,
diluted 1/5
1/50
1/250 in saline.

The chambers were then filled with TC199 containing 10% horse serum, and sealed with round coverslips.

HSV antigen for the tests was prepared in the following way:

Confluent monolayers of Vero tissue culture cells were infected with herpes virus and incubated at 36°C until full cytopathic effect was evident. The infected cells were harvested into the medium maintaining the cells, then centrifuged at 2000 rev/min for 10 min. The pelleted cell debris was resuspended in peptone water equal to 1/10 of the original volume of harvested material. The cells were disrupted by sonication at 20 Kc/s (MSE Ultrasonic Disintegrator—100 watt model). The virus was inactivated by treatment with 0·3% *β*-propiolactone. No preservative was added. Control antigen was prepared in the same way from uninfected Vero cells grown in parallel with the infected cells.

The antigen contained 10·5 mg/ml of protein and 1·2 mg/ml of nucleic acid. The control solution contained 8·1 mg/ml protein and 0·9 mg/ml nucleic acid. These estimations were made spectrophotometrically. The chambers were filled with TC199 + 10% horse serum, closed with round coverslips and incubated for 18–24 h at 37°C.

Measurements of the migration area were carried out using a microscope carrying a projection attachment. The areas were traced and measured using a planimeter.

The migration index was calculated as:

$$\frac{\text{average migration area with antigen}}{\text{average migration area with control antigen at the same dilution}}$$

$$\text{With PHA the ratio of } \frac{\text{average area with PHA}}{\text{average area with normal saline}} \text{ was taken.}$$

Complement fixation test

The test was carried out on batches of sera stored at −40°C. Herpes simplex complement-fixing antigen prepared by the Standards Laboratory for Serological Reagents at Colindale, and guinea-pig complement (Wellcome) were used. The standard technique of Bradstreet & Taylor (1962) was followed.

Patients with a history of one or more attacks of herpes simplex per year were chosen. All except one had lip lesions, the exception having lesions near the knee. Control subjects were healthy hospital staff.

RESULTS

Leukocyte migration test

Migration indices with undiluted herpes simplex antigen were significantly less in control subjects than in herpes patients (Fig. 1) This difference was not present when diluted antigen was used. There was no difference between the two groups in the response to PHA at the concentration used.

A migration index of 0·85 or less has been accepted as indicating significant inhibition. Taking this

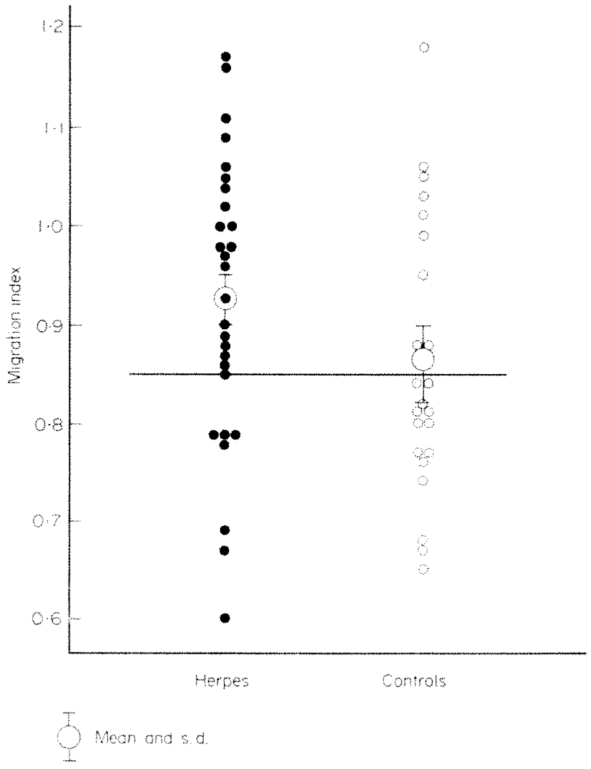

FIGURE 1. Migration indices in herpes patients and control subjects.

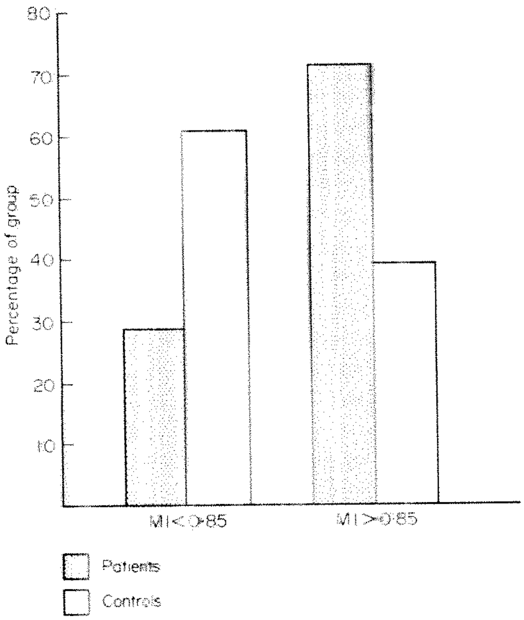

FIGURE 2. Distribution of migration indices.

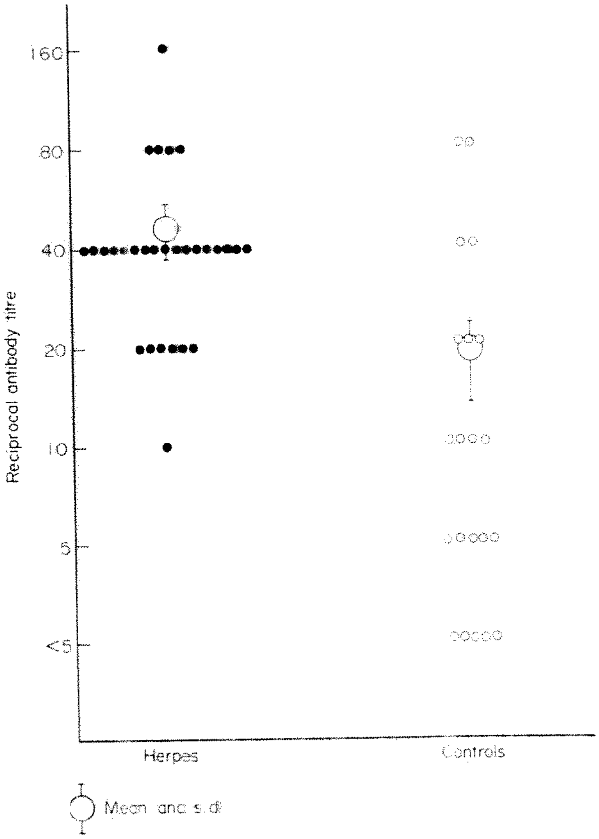

FIGURE 3. HSV antibody levels.

limit, the difference between patients and control subjects is highly significant, 71% of controls but only 28·5% of herpes subjects falling into this group ($\chi^2 = 5{\cdot}588$; $P = 0{\cdot}05$) (Fig. 2).

If patients were divided into those with active or recent lesions and those without recent activity, there was no difference between their test results.

Complement fixation test

All herpes subjects had measurable levels of circulating antibody to herpes simplex virus; in only one patient was the titre less than 1/20 (Fig. 3). In control subjects the levels were much lower, 67% having titres less than 1/20, the difference being highly significant ($P = 0{\cdot}0005$).

DISCUSSION

That humoral antibodies alone play no significant part in resistance to recurrent HSV lesions has been established (Douglas & Couch, 1970).

Laboratory studies, including our own, suggest that an altered cellular response to HSV antigen is present in herpes subjects. Using guinea-pig macrophage inhibition, Wilton *et al.* (1972) demonstrated that only 7·1% of herpes subjects respond to antigen compared with 39% of the controls. Lymphocyte toxicity studies also showed less activity in herpes subjects than in controls. Using lymphocyte transformation there was no significant difference. Russel (1974) found, however, that lymphocyte transformation by HSV antigen occurred in all herpes subjects but only in four out of thirty-seven controls.

In an experimental situation, using rabbits immunized with HSV, Rosenberg, Farber & Notkins (1972) found a thirtyfold increase in transformation of splenic lymphocytes by inactivated HSV, which was not inhibited by prior incubation of the virus with the antibody.

In experimentally immunized guinea-pigs, delayed hypersensitivity to HSV, measured by skin testing, could be passively transferred by cells but not by serum (Lausch, Swyers & Kaufman, 1966).

Dissociation of different measurements of cellular immunity is well recognized (Bloom, Gaffney & Jimenez, 1972) and is not necessarily contradictory. In chronic mucocutaneous candidiasis, migration inhibition factor (MIF) production may occur in the absence of transformation or the reverse (Kirkpatrick, Rich & Smith, 1972). In pollen allergy, transformation may occur without MIF production. In sensitization to tobacco mosaic virus antigen derivatives, some substances could produce positive skin tests and MIF production but failed to produce transformation (Spitler *et al.*, 1970). It seems likely that MIF production occurs either by a separate pathway or as a step in the sequence of events which culminate in blastogenesis (Chaparas *et al.*, 1970). In culture, MIF is demonstrable after 2 h incubation whereas morphological changes do not occur before 20 h. Vinblastine and cytosine arabinoside, while greatly reducing cell division, do not interfere with MIF production *in vitro* (Bloom *et al.*, 1972). The effector mechanism in delayed hypersensitivity is probably associated with a mature dividing cell.

The peripheral leukocyte migration inhibition test, while it may not be a measure of MIF production, has been accepted as a measure of cell mediated immunity and correlates well with delayed hypersensitivity and skin testing (Bloom *et al.*, 1972; Søborg & Bendixen, 1967).

The use of the test in our study supports the concept of a localized defect in the effector limb of cellular immunity, probably concerned with the production of MIF or a related substance.

ACKNOWLEDGMENTS

We would like to thank the South West Thames Regional Hospital Authority for their financial support and Dr B. Watson, for his help with the statistics.

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British Journal of Dermatology (1975) **93**, 545.

In vitro assay of immunity to human wart antigen

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Accepted for publication 3 March 1975

SUMMARY

The cell-mediated and antibody responses to human wart antigen have been studied in patients with warts and in controls. Most patients show a positive cell-mediated response at or about the time of resolution of their warts but the detectable immunity is short-lived. The majority of patients with warts have antibodies and the percentage rises both with resolution of the warts and with increase in the duration of the infection. No correlation between the presence of antibodies and the development of cell-mediated immunity was found.

Preliminary results on a small group of patients with warts, using an *in vitro* assay of cell-mediated immunity to human wart antigen, have recently been published (Morison, 1974). The study has now been extended to a greater number of patients and in addition the humoral response to the same antigen has been investigated. The aim of this dual study was to characterize further the mechanisms involved in the resolution of wart virus infections.

PATIENTS AND CONTROLS

Patients

118 patients were examined and they fell into three categories: Fifty-two patients were examined on two or more occasions, at least once while their warts were judged, clinically, to be active. Five patients were examined only after resolution of their warts. Sixty-one patients were examined only before resolution of their warts. There were sixty-two females and fifty-six males with a mean age of 30 years and a range of 17–72 years. Treatment was prescribed at the first attendance, the procedures used being: paint applied daily, cryotherapy or excision. The five patients examined only after resolution of their warts had not had any treatment.

Control subjects

Forty-one healthy people were examined but of these only twenty-eight had cell-mediated immune studies. There were nineteen males and twenty-two females with a mean age of 48 years and a range of 18–82 years. Ten had a past history of warts but none within the previous 5 years.

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METHODS

Studies of cell-mediated immunity

As previously reported (Morison, 1974) the leukocyte migration inhibition test (LMI test) was used to assess the *in vitro* response to a human wart antigen (HWA). Unless otherwise stated the latter was used in a dilution of 10^{-3} for all aspects of the present study. Two further antigens were also tested:

Crude wart antigen. A portion of the concentrated HWA was diluted to 10^{-3} using the supernatant removed after grinding the warts in acid-washed sand. Thus this antigen contained skin and serum proteins from many individuals.

Individual wart antigen. Individual antigens were prepared from their own warts in some patients, scrapings being removed from their warts at their first attendance.

Studies of humoral immunity

A control positive serum was prepared by inoculation of a rabbit with HWA. The strength and specificity of the antiserum were checked by the method of double immuno-diffusion (Ouchterlony, 1958) and in addition the specificity was checked by electron-microscopy using the negative staining technique (Almeida & Goffe, 1965) after adsorption by normal skin. The pre-inoculation rabbit serum was used as a negative control. Serum was obtained from patients and controls at the time of performing the cell-mediated immune studies and stored at −70° C.

Methods for antibody detection

Two test systems were used. The first was counter-current immuno-electrophoresis on cellulose acetate membrane (CIE), being based on the technique of Pesendorfer, Krassnitsky & Wewalka (1970). Test sera in 2 μl volumes were placed 1 cm apart along a 50×360 mm cellulose acetate membrane and opposite, HWA in the same volume was placed at a distance of 6 mm. Electrophoresis was carried out at a gradient of 100 V for 30 min after which the membrane was washed and stained. A positive result is indicated by a line of precipitation at the site of contact of antigen and antibody. For all tests controls of positive and negative rabbit antiserum were run.

The second system was that of haemagglutination inhibition (HI) based on a technique described by Fulthorpe (1957). The method involves adding HWA to the test serum and after 12 h fresh sheep red cells, sensitized with rabbit antiserum, are added. Haemagglutination indicates that antibody is not present in the patients' serum in a detectable quantity. For the procedure the immunoglobulins in the rabbit antiserum were precipitated by the ammonium sulphate technique (Weir, 1967) and used in a volume of 1/5 of the original volume of serum. Two samples of red cells were sensitized, one with the immunoglobulin suspension and the other with normal rabbit serum, glutaraldehyde being used as the coupling agent (Onkelinx *et al.*, 1969). Test sera were decomplemented and then adsorbed with the serum-tagged cells to remove heterophile antibodies to red cells or rabbit serum. Both lots of sensitized and unsensitized cells were included in all tests as controls and only a complete smooth carpet of cells spread evenly over the bottom of the well was taken as positive for haemagglutination, degrees of haemagglutination less than this being regarded as negative and therefore indicating the presence of antibody to HWA in the test serum. The HWA used for this test was frozen and thawed 40 times and used in a dilution of 1:80. Preliminary experiments showed that progressive freezing and thawing of the antigen greatly increased its strength permitting an 8-fold dilution after freezing and thawing 40 times.

Immunoglobulin determinations

Sera from patients positive on HI were examined further to determine the immunoglobulin class responsible for the positive result. Three treatments were used for the successive removal of IgM, IgG and IgA. The effectiveness of each treatment in removing the particular protein was first assessed on normal sera and on sera containing an excess of the protein, these sera then being tested for residual Ig by means of two-dimensional immuno-electrophoresis (Minchin Clarke & Freeman, 1968).

Firstly mercaptoethanol was used on all sera to destroy IgM. Serum was incubated with a 1:10 volume of 0·2 M 2-mercaptoethanol at 37°C for 2 h and the serum was then tested in the HI system. Next IgG was removed using staphylococcal protein A adsorption. This procedure is based on an observation that certain strains of *Staph. aureus* produce a protein, termed protein A, which reacts with the Fc part of IgG globulins and that three of the four sub-classes of IgG can be precipitated by this protein (Jensen, 1958; Forsgren & Sjoquist, 1966; Kronvall & Williams, 1970). *Staph. aureus*, phage type 52 (NCTC No. 8530), was used for the procedure; test sera were adsorbed using a 1:2 dilution of packed bacteria and incubating at 37°C for 30 min. Sera from patients still positive after the mercaptoethanol treatment were adsorbed using this technique. Two serum samples from each patient, original untreated serum and post-mercaptoethanol treated serum, were then tested in the HI system. Sera still positive after these last two treatments were further adsorbed using anti-human IgA serum (Wellcome) in a dilution of 1:8 for 1 h at room temperature. Three serum samples from each patient, original untreated serum, post-mercaptoethanol serum and post-staph. protein A serum, were adsorbed with the anti-human IgA serum and tested in the HI system for antibody activity to HWE.

RESULTS

Cell-mediated immunity

As mentioned in the previous study (Morison, 1974), a migration index of less than 0·80 was regarded as a positive result. The results obtained in the fifty-seven patients examined at least once after resolution of their warts are shown in Table 1. Of these patients nine showed a positive response to wart antigen at the first examination, of whom eight were clear of their warts within 2 months. Apart from this there was no correlation between duration of treatment and degree of responsiveness to wart antigen. The results obtained in the pre-resolution tests in patients having a single examination were compared with those in patients having two or more examinations but there were no significant differences between these two populations as judged by the single examination. The responsiveness to

TABLE 1. Results of cell-mediated immune studies

Treatment	Number examined	Number of patients showing an increased and positive response in LMI test after resolution
Excision and cautery	8	1
Cryotherapy	12	7
Paint	32	29
Nil	5	5

wart antigen was followed in four patients who gave a positive response in their first post-resolution examination. All had lost their positive response within 10–13 weeks.

Six patients were examined both before and after resolution of their warts using both HWA and crude wart antigens for comparison. There was some minor variation in the results but in no case did a patient show a conversion from a negative to a positive response by using the crude wart antigen. Nine patients were examined using their own wart antigen in comparison with HWA and in four examination was possible both before and after resolution of their warts. There were no marked variations in the results and if patients were positive with HWA they were positive with their own antigen and vice versa.

None of the twenty-eight controls gave a positive response to wart antigen.

Humoral immunity

The rabbit antiserum was found to contain a specific antibody to HWA both by the Ouchterlony technique and by electron-microscopy, there being no reaction with an extract of normal skin and no alteration in strength after adsorption with normal skin.

The results of the antibody studies are shown in Table 2. It can be seen that a much larger percentage of patients were positive using the HI system as compared with the CIE test and that the

TABLE 2. Results of antibody studies using haemagglutination inhibition (HI) and counter-current immuno-electrophoresis (CIE)

	Number of patients	% Positive on HI	% Positive on CIE	Total % positive
A. Patients examined at least once after resolution of warts				
Pre-resolution	52	67	21	77
Post-resolution	57	82	47	88
B. Patients examined only before resolution	61	70	28	70
C. Control subjects	41	37	17	37

TABLE 3. Types of immunoglobulin response to HWA

	Number giving positive result	% Distribution antibody classes			
		IgM	IgG	IgM + IgG	IgM + IgG + IgA
A. Patients examined at least once after resolution of warts					
Pre-resolution	35	78		22	
Post-resolution	47	57	13	21	9
B. Patients examined only before resolution	43	70	2	23	5
C. Control subjects	15		100		

number having antibodies increased after resolution of the warts. A number of patients were positive by the HI system and negative with CIE and vice versa. The results of the immunoglobulin determinations are shown in Table 3 and it can be seen that pre-resolution sera showed mainly an IgM response while post-resolution sera contained IgG or IgA in almost 50 % of cases. The results obtained with the HI system on pre-resolution sera were analysed according to the length of history of warts. The results are shown in Table 4 and indicate that patients with a history exceeding 3 years had antibodies more frequently than those with a history of less than a year and that the proportion of patients having IgG and IgA antibodies rose with the duration of the infection. However a history of 1–3 years was associated with a lower incidence of antibodies. Since the type of treatment appeared to correlate with the cell-mediated immune response, the effect of this on antibody response was analysed but no pattern was evident.

TABLE 4. Relationship of antibody response to duration of wart infection

Duration of wart infection in years (No. patients in brackets)	% Patients with detectable antibody (No. in brackets)	% Distribution of antibody classes			
		IgM	IgG	IgM + IgG	IgM + IgG + IgA
< 1 (41)	68 (28)	96		4	
1–3 (38)	53 (20)	85		15	
> 3 (34)	88 (30)	48	3	43	6

Of the ten controls with a past history of warts eight had detectable antibodies. Of the thirty-one controls without a history of warts seven had detectable antibodies.

Comparison of humoral and cell-mediated studies

Antibody results as measured by both systems were compared to the cell-mediated immune responses and the analysis is shown in Table 5. Patients examined pre- and post-resolution showed no correlation

TABLE 5. Relationship of antibody response to cell-mediated immune response

	Number of patients showing positive response to CWA in LMI test	% Column 1 patients with detectable antibody	Number of patients showing negative response to CWA in LMI test	% Column 3 patients with detectable antibody
A. Patients examined at least once after resolution of warts				
Pre-resolution	9	77	43	77
Post-resolution	42	87	15	90
B. Patients examined only before resolution	10	60	51	72

between the two responses. Patients examined only before resolution who showed a negative response in the LMI test were more likely to have antibodies as compared to those showing a positive response. However the number in the latter group was small.

DISCUSSION

The present study reinforces the earlier finding that cell-mediated immunity, as measured by the LMI test, to human wart antigen increases in some patients at the time of resolution of their warts. Unfortunately the ideal study could not be done, namely to follow a group of patients without using any treatment. However by using several treatments some ideas can be put forward. Excision and cautery do not appear to alter immunity and that is probably due to the antigen being separated, rapidly, away from the host, preventing any change in immune status. Successful cryotherapy probably does the same but some patients having this treatment presumably develop an inflammatory reaction sufficient to allow their lymphocytes to become sensitized to wart antigen. The situation is different when a paint is used as treatment. Almost all patients showed an increased and positive response to wart antigen. The three who did not were possibly examined at the wrong time, immunity waning by the time of the examination. That the increase in immunity seen in most patients is not likely to be just a side-effect of treatment is shown by the positive response found in the five patients whose warts underwent spontaneous resolution without any treatment. Furthermore, that a positive response is an integral part of successful resolution is indicated by the fairly rapid resolution of the warts in the nine patients having a positive response at the first examination.

The duration of the immune response as tested by this system is short, not extending beyond 3 months in any of the patients tested serially. This might explain why a further attack of warts can occur only months after one has resolved.

It is not possible to grow wart virus reliably and therefore extracts of warts have to be used as antigen; this raises the possibility that the test is measuring sensitivity to other skin and serum proteins in the pooled antigen. By using the crude wart antigen a deliberate attempt was made to increase the quantity of skin and serum proteins in the antigen. This did not result in any of the patients tested converting from a negative to a positive response. Furthermore, when some patients were tested with their own antigen there were no conversions. Although the possibility of sensitivity to other than wart antigen is not completely excluded it would appear unlikely. Sensitivity to the wart virus as opposed to sensitivity to other wart antigens is not established in this study and this point will only be clarified when the wart virus is grown in culture.

A number of systems have been applied to the study of the antibody response to wart antigen in humans with as many different results. For the present study the CIE system was first tried but, as previous workers have found (Cubie, 1972), it is relatively insensitive. The HI system was therefore tried and while this system is not efficient in the quantitative evaluation of the antibody response it does appear to give a good qualitative assessment. Of the patients with active wart infection, 73% had antibodies and the predominant antibody was IgM, it being the only antibody present in 73%. Sera obtained from patients after resolution of their warts showed an increased incidence of antibodies, 88%, and while IgM is still the dominant antibody, 43% also contain IgG. Analysis of results in terms of length of history of warts shows that the longer the warts are present the more likely are antibodies to develop, 68% of patients with a history shorter than a year having antibodies as compared with 88% of those with a history of 3 years or longer. In addition only 4% of patients with a history of less than a year had IgG antibody while 52% of those with a history of 3 years or more had this antibody. It can therefore be concluded that both resolution of warts and increasing duration of warts are associated with an increased incidence of antibodies and the development of antibodies in the IgG class. It has been shown in guinea-pigs that a small dose of wart antigen elicits only an IgM response while a larger dose produces an IgM and an IgG response (Furminger, 1970). Wart virus, being mainly in the upper layers of the epidermis (Almeida, Mowatson & Williams, 1962), probably does not reach antibody-forming cells in very large quantities. However spontaneous resolution, inflammation associated with treatment and accidental trauma no doubt release a larger amount of antigen thus explaining the alteration in the antibody status.

Among the control subjects, 37% had antibodies to wart antigen, all of the IgG class. Half of the controls with antibodies did not give a past history of warts. Similar results have been reported previously (Ogilvie, 1970).

This study has failed to demonstrate any positive correlation between the development of cell-mediated immunity and the presence of antibodies. Resolution of warts is associated with an increase in the incidence of antibodies but a similar increase was seen in patients who developed cell-mediated immunity as compared with those who did not. Whereas there is a close correlation between the method of treatment and the development of cell-mediated immunity there is no indication that the method of treatment influences the type or presence of antibody. It would appear that cell-mediated immunity to wart antigen is closely associated with the resolution of warts but whether it is a cause or effect relationship is not established in this study.

ACKNOWLEDGMENTS

Dr June Almeida kindly performed the electron-microscopy in this study and Mr David Borrett cultured the bacteria for the adsorption technique. The details of the protein A adsorption were supplied by Dr John Pattison who suggested the technique. Dr E.L.Rhodes kindly permitted study of her patients and has greatly helped with encouragement and comment. I also thank my colleagues for their co-operation in collecting samples of warts. The study has been made possible by a grant from the South West Metropolitan Regional Hospital Board.

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British Journal of Dermatology (1975) **93**, 553.

Cell-mediated immune responses in patients with warts

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Accepted for publication 3 March 1975

SUMMARY

Competence of cell-mediated immunity in patients with warts was assessed by measuring *in vitro* response to PHA and PPD. Patients with warts were found to be less responsive as compared with a control group and this deficiency was related to the length of history of the wart infection.

The suggestion has been made that cell-mediated immune responses play a part in achieving successful resolution of wart virus infections (Morison, 1974a, 1975). If that is so, then possession of a competent cell-mediated immune system would be a prerequisite for the successful resolution of warts in a patient. Some patients have difficulty in eradicating their warts and perhaps they are different from a normal population, either in respect of their cell-mediated immune function in general or only as it applies specifically to wart antigen. The aim of the present study was to test the first possibility by comparing the cell-mediated immune responses in a group of patients with warts to those of a control group. Two tests of such responses were studied, response to purified protein derivative (PPD) and response to phytohaemagglutinin (PHA), both being tested *in vitro*.

PATIENTS AND METHODS

Patients

A total of seventy-eight patients were investigated, thirty-eight females and forty males, with a mean age of 30 years and a range of 17–71 years. All patients, apart from having warts, were considered to be fit and had no significant systemic disease. The duration of wart infection varied; twenty-seven patients gave a history of less than 1 year, twenty-two patients more than 1 but less than 3 years and twenty-nine patients gave a history of more than 3 years.

Control subjects

Sixteen healthy control subjects were examined, eight males and eight females with a mean age of 43 years and a range of 24–74 years.

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Methods

Cell-mediated immune responses to the antigen and mitogen were measured using the leukocyte migration inhibition test (Søborg & Bendixen, 1967). PPD (Weybridge, freeze-dried) was used in a concentration of 100 μgm/ml in activated chambers. This concentration was found satisfactory for distinction between Mantoux positive and negative subjects as has been described (Rosenberg & David, 1971). A migration index less than 0·80 indicates sensitivity to the antigen. PHA (Wellcome, freeze-dried grade) was added to activated chambers in a concentration of 5 μgm/ml. A previous study (Morison, 1974b) indicated that with this mitogen a migration index above 0·50 is abnormal, between 0·30 and 0·50 is equivocal and below 0·20 is normal.

RESULTS

PHA stimulation in patients

The results obtained are shown in Fig. 1. Eleven patients (14·1%) had results above an index of 0·50 and twelve patients (15·3%) had an index between 0·30 and 0·50. The mean result for the group was 0·26 with a range of 0·03–0·76. Table 1 shows the results subdivided according to the duration of the infection. The mean migration index is higher in patients with a longer history of warts and, in addition, almost all patients with migration indices greater than 0·30 had had warts for longer than 1 year. Those patients with warts for longer than 3 years had a mean index above 0·30 and over 50% had a result above 0·30.

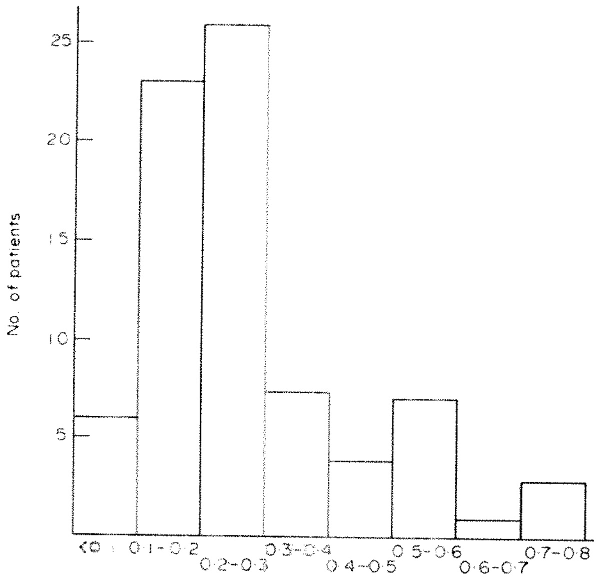

FIGURE 1. Migration indices following PHA stimulation in patients with warts.

Suppression of the PHA response by the wart infection did not appear to occur; nine patients were examined both before and after the resolution of their warts and the greatest difference between the two results was 0·06.

TABLE 1. Results of PHA and PPD stimulation analysed according to duration of wart infection

Duration of wart infection (years)	Number of patients	Mean migration index on PHA stimulation	% with index >0.30 on PHA stimulation	% with negative response to PPD
<1	27	0·19	4	7
1–3	22	0·25	32	41
>3	29	0·36	52	31

PPD stimulation in patients

Twenty of the seventy-eight patients (26 %) gave a negative result. It can be seen from Table 1 that most of these patients had a history of warts exceeding 1 year.

Control subjects

With PHA stimulation the mean migration index was 0·15 with a range of 0·03–0·27. On PPD stimulation two (13 %) gave a negative result.

A summary of the results obtained in patients and control subjects is given in Table 2.

TABLE 2. Summary of PHA and PPD results

	Patients with warts	Control subjects
Mean migration index on PHA stimulation	0·26	0·15
% with index >0·30 on PHA stimulation	29	0
% with negative response to PPD	26	13

DISCUSSION

The patients in the present study are not representative of people with warts in the general population as there is a strong bias towards those who have difficulty in eradicating their infection. For this reason they are suitable for the present study. The responses to PHA among the patients are outside the normal range in 29 % of cases whereas, by contrast, none of the controls exceeded this range. This abnormality bears a strong relationship to the duration of the warts, patients with warts of short duration giving a normal result in almost all cases, while patients with a long history giving an abnormal result in over 50 % of cases. The responses to PPD also appear to be different in patients as compared with controls. However it is realized that these are not matched groups. The PPD results also correlate with the duration of the infection; most of the patients with a negative result had had their warts for more than 1 year.

There appears to have been only one previous study of the immunological make-up of patients with warts (Brodersen, Genner & Brodthagen, 1974). It was found that children with warts had a significantly lower incidence of positive tuberculin skin tests as compared with a control group. The possibility that the viral infection suppresses tuberculin sensitivity was not investigated but the present *in vitro* study would indicate that this is not a significant factor.

Warts are a very common infection and it would seem probable that in general they develop not because of any significant immune or other deficiency, but rather as a result of exposure and change. However there is marked variation in patient ability to control and eradicate the infection and some patients have what appear to be very resistant warts. A problem in the present study has been to define a 'resistant' wart. Duration of infection has been selected but it is realized that number of warts and amount of treatment are other factors which could be considered before deciding that warts are resistant. However accepting these limitations the present study indicates that non-specific cell-mediated immune responses are to some extent defective in patients with a long history of wart infection. It therefore is postulated that the lack of a fully competent cell-mediated immune system may well be the reason why such patients have difficulty in eradicating their warts.

ACKNOWLEDGMENTS

Dr E.L.Rhodes kindly permitted examination of her patients. This work has been made possible by a grant from the South West Metropolitan Regional Hospital Board.

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British Journal of Dermatology (1975) **93**, 557.

Nickel hypersensitivity

NICKEL BINDING TO AMINO ACIDS AND LYMPHOCYTES

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Accepted for publication 9 February 1975

SUMMARY

The nature of a protein hapten conjugate which could effect lymphocyte transformation in nickel hypersensitivity was investigated by subjecting the culture media employed to radiochromatographic analysis. Autoradiographic studies with ^{63}Ni demonstrate direct binding of nickel salts to the lymphocyte cell surface. This occurs with a proportion of lymphocytes both from nickel sensitive and from control subjects and so binding is not of itself the stimulus for transformation.

We have previously shown (Hutchinson, Raffle and MacLeod, 1972) that in nickel sensitive subjects positive lymphocyte transformation tests are obtained when nickel is used as a stimulant in optimum concentrations, either as the sulphate or as the acetate, and that this nickel induced transformation is specific for subjects who show clinical and patch test evidence of nickel sensitivity. These findings have been confirmed by other workers (Forman & Alexander, 1972; Millikan, Conway & Foote, 1973).

The antigenic potential of nickel salts is manifest with lymphocytes in the presence of Medium 199. In an attempt to elucidate the binding of nickel in this system we have carried out radiochromatographic and autoradiographic studies.

Radiochromatography

The following radiochromatographic studies were carried out:

Preliminary 2-way thin layer cellulose chromatograms with Medium 199 to which ^{63}Ni as $NiCl_2$—Sp. act. 1 mCi/ml (95 μg Ni/ml)—was added showed that the nickel had been bound to glycine, histidine, lysine, and to a lesser extent to aspartic acid and serine. 30 μl of ^{63}Ni ($\equiv 10^{-4}$ mEq Ni/ml) were subsequently added to 1 ml of each of the following solutions:

(1) L-Lysine monohydrochloride (1 mg/ml sterile water),
(2) L-Histidine (1 mg/ml sterile water),
(3) Glycine A.R. (1 mg/ml sterile water),
(4) Medium 199.

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Plates were run in respect of each, both for qualitative assay by autoradiography and quantitative assay by liquid scintillation counting of the individual amino acid spots.

Autoradiography

^{63}Ni solution was added to cultures of control lymphocytes and of nickel sensitive lymphocytes, suspended in Medium 199 or in phosphate buffered saline (PBS) with or without the addition of autologous serum, and autoradiography subsequently performed. The lymphocytes (> 90 % of cell content) were obtained by Ficoll Triosil separation (Harris & Ukaejiofo, 1970) from venous blood of 3 nickel sensitive and paired control subjects and ^{63}Ni was added as follows to duplicate culture tubes:

(1) WBCs suspended in 1·5 ml Medium 199 plus 0·5 ml autologous serum plus 60 μCi ^{63}Ni ($\equiv$ 10^{-4}m Eq Ni/ml),

(2) WBCs suspended in 1·5 ml PBS plus 0·5 ml autologous serum plus 60 μCi ^{63}Ni,

(3) WBCs washed x 3 with PBS plus 2 ml PBS plus 60 μCi ^{63}Ni.

Sample (1) was harvested after 6 days while samples (2) and (3) were harvested after 24 h. Autoradiography was carried out using Kodak A.R.10 stripping film which was left in contact with the cell smear for 6 weeks before developing. The smears were counter-stained with Giemsa stain.

RESULTS

Radiochromatography

Radiochromatography identified nickel binding to lysine, histidine, glycine, serine, and aspartic acid, in the Medium 199. The binding was in the ratio of 47 : 3·5 : 2 : 1 : 1. These figures were confirmed by the results for the individual amino acids which again showed lysine to be the major binding amino acid.

Autoradiography

Autoradiographs were obtained and these showed binding of ^{63}Ni to approximately 20 % of lymphocytes both from normal and from nickel sensitive subjects (Figs 1 and 2). Binding occurred not only with cells suspended in Medium 199 and cells suspended in serum but also with cells suspended in phosphate buffered saline.

DISCUSSION

Antigenic triggering of lymphocytes sensitized to nickel may occur in one of three ways. Nickel may form a conjugate with amino acids or proteins in the serum, it may bind directly to lymphocytes, or it may react with serum components to produce some unknown factor which is the sensitizer. Our results show that an amino acid conjugate is formed, mainly with lysine, in the culture medium, and other workers have shown that a nickel protein complex can be isolated from human serum (Himmelhoch *et al.*, 1966). Mirza, Perera & Bernstein (1974) have reported positive results with a leukocyte migration inhibition test in nickel dermatitis using a nickel bovine albumin complex. Our results also show that nickel binds directly to the lymphocyte cell surface of approximately 20 % of lymphocytes from both normal and nickel sensitive subjects, irrespective of the supporting medium. Hence the conditions exist for at least two of the possible triggering mechanisms and it may be that hapten conjugation occurs on the cell surface. This possibility is also raised by the results of other workers. Geczy & Baumgarten (1970) have concluded, as the result of lymphocyte transformation and autoradiographic studies, that the conjugation of dinitrofluorobenzene (DNFB) to the lymphocyte

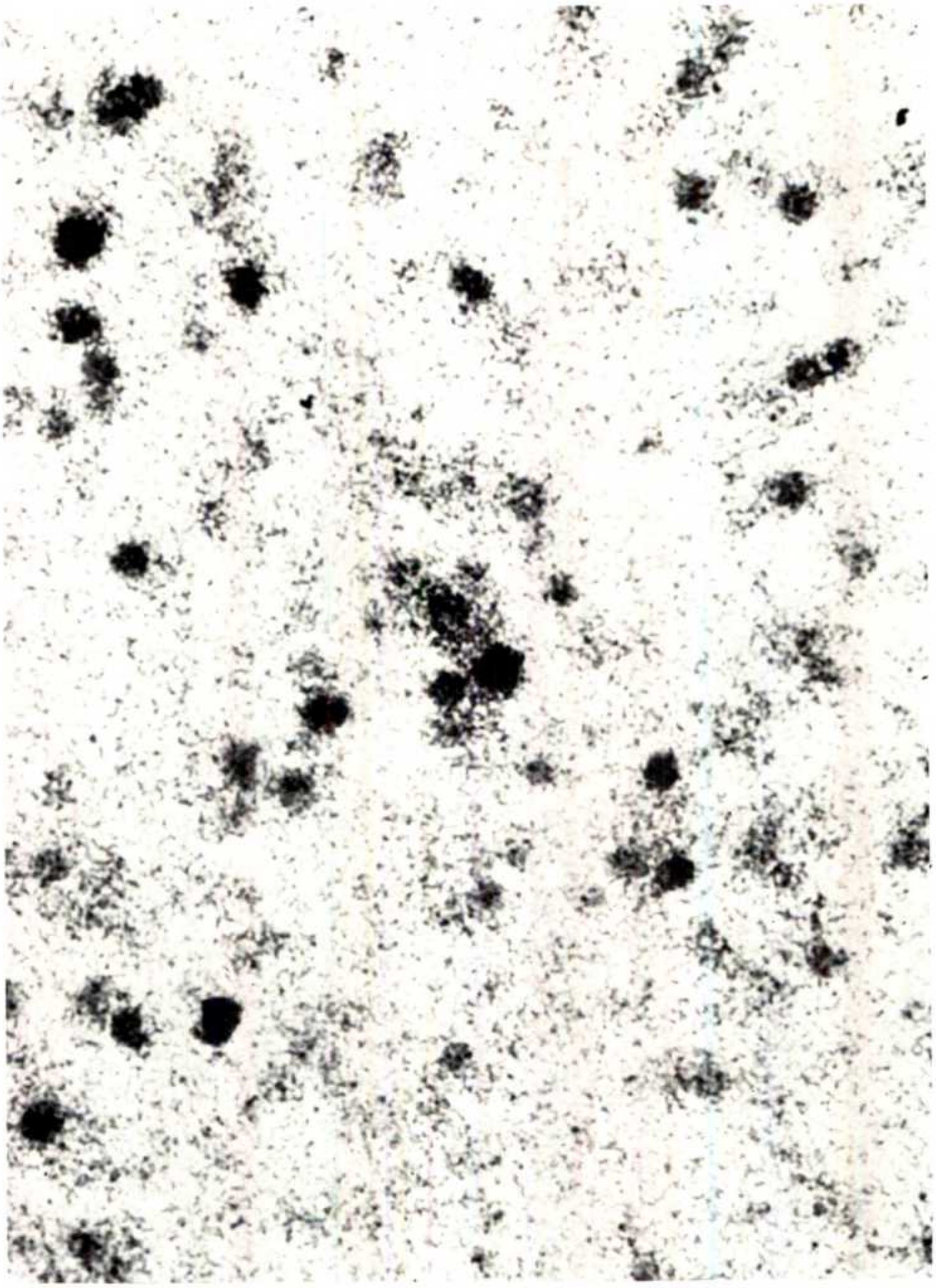

FIGURE 1. Autoradiograph showing binding of ^{63}Ni to lymphocytes (× 500).

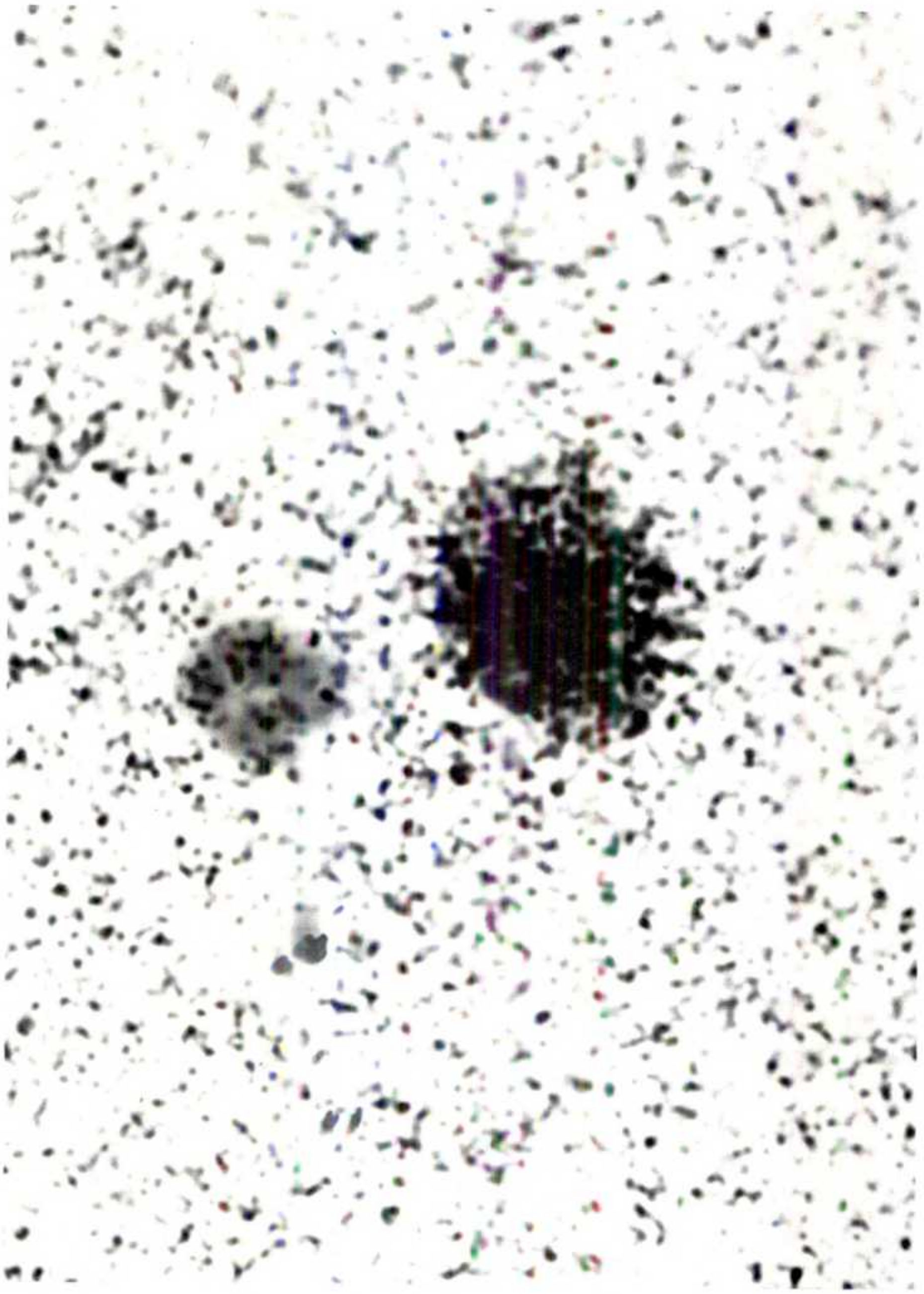

FIGURE 2. Autoradiograph showing binding of ^{63}Ni to lymphocyte (× 2000).

triggers transformation in guinea-pigs sensitized to this chemical. McFarlin & Balfour (1973) were able to induce contact sensitivity in the pig against DNFB by *in vitro* conjugation of Dinitrophenyl to autologous lymph node cells and subsequent infusion via afferent lymphatics. Jones & Amos (1974) adduce evidence that beryllium sulphosalicylate will react directly with guinea-pig lymphocytes and trigger them to produce migration inhibition factor (MIF). Hence it may well be a general phenomenon that hapten protein conjugation occurs on the surface of the lymphocyte.

However, the labelling of lymphocytes with ^{63}Ni, which occurs in approximately 20% of both the nickel sensitive and control subjects, is different from that in the case of ^{203}Hg (Carron, Poutala & Provost, 1970) which showed no specific cellular labelling. Nickel however is a transition element which has been shown to be a specific stimulant, and the different electronic configuration of the heavy metal mercury which is a non-specific stimulant may account for this. The nickel labelling may relate simply to the cell membrane characteristics of a sub-population of peripheral lymphocytes and be analagous to the binding of a saccharose-Fe-III-hydroxide glycine compound (FHG-S) to lymphocytes demonstrated by Desaga (1974) where different binding patterns occur with T and B cells.

Further work is obviously required in order to elucidate the basic mechanism of nickel hypersensitivity.

ACKNOWLEDGMENT

We wish to acknowledge the help of Mr D. Donald in this work.

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British Journal of Dermatology (1975) **93**, 563.

Pharmacology and Treatment

Comparative bio-availability and activity of proprietary topical corticosteroid preparations: vasoconstrictor assays on thirty-one ointments

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Accepted for publication 5 March 1975

SUMMARY

Thirty-one proprietary corticosteroid ointments were evaluated for vasoconstriction using an occluded blanching test. The results were expressed as three parameters: (1) area under the blanching curve, (2) summed % total possible score, and (3) square root transformation of sum of scores divided by number of volunteers. Experiments were performed to (a) compare the bio-availability and activity of the ointments, (b) determine the effect of antimicrobial additives, and (c) assess the retention of steroids in the skin. Scores for the additive-containing and 'plain' preparations were within 90% except for Locorten-Vioform/Locorten (58%). A steroid reservoir in the skin was demonstrated lasting for 8–14 days. Selected preparations were re-tested by a non-occluded method. The results for the ointments were compared with those previously obtained using the corresponding creams.

Barry & Woodford (1974) compared the bio-availability and activity of thirty proprietary corticosteroid creams and gels using the vasoconstrictor assay. That work has now been extended to the corresponding ointments.

MATERIALS AND METHODS

Thirty-one topical corticosteroid ointments (Table 1) were investigated, the volunteers and methods employed being as previously described (Barry & Woodford, 1974).

RESULTS AND DISCUSSION

The blanching response for each ointment is a measure of its activity as a function of time. The 'activity' of a preparation is its ability to produce pallor as reflected by the area under the blanching curve; hence, corticosteroid formulations may be ranked according to their activity, irrespective of the steroid or base employed. The 'bio-availability' of a preparation refers to the activity of a given corticosteroid in a particular base compared to its activity in different bases. Thus, the bio-availability and

TABLE 1. Proprietary ointments used in vasoconstrictor assays

Ointment	Manufacturer	Steroid and % w/w	Additive and % w/w
Adcortyl	Squibb	Triamcinolone acetonide, 0·1	—
Betnovate	Glaxo	Betamethasone as valerate, 0·1	—
Betnovate-A	Glaxo	Betamethasone as valerate, 0·1	Aureomycin, 3·0
Betnovate-C	Glaxo	Betamethasone as valerate, 0·1	Clioquinol, 3·0
Betnovate-N	Glaxo	Betamethasone as valerate, 0·1	Neomycin sulphate, 0·5
Deflamene	Pharmitalia	Formocortal, 0·025	—
Deflamene-A	Pharmitalia	Formocortal, 0·025	Paromomycin sulphate, 0·5
Dermovate	Glaxo	Clobetasol propionate, 0·05	—
Drenison	Lilly	Flurandrenolone, 0·05	—
Drenison with Neomycin	Lilly	Flurandrenolone, 0·05	Neomycin sulphate, 0·5
Ficoid-2	Fisons	Fluocortolone pivalate, 0·1 Fluocortolone hexanoate, 0·1	—
Ficoid-2 Plus	Fisons	Fluocortolone pivalate, 0·1 Fluocortolone hexanoate, 0·1	Clemizole hexachlorophenate, 2·5
Haelan	Dista	Flurandrenolone, 0·0125	—
Haelan-C	Dista	Flurandrenolone, 0·0125	Clioquinol, 3·0
Hydrocortistab	Boots	Hydrocortisone acetate, 0·1	—
Hydrocortisyl	Roussel	Hydrocortisone, 0·1	—
Ledercort	Lederle	Triamcinolone acetonide, 0·1	—
Locorten	Ciba	Flumethasone pivalate, 0·02	—
Locorten-Vioform	Ciba	Flumethasone pivalate, 0·02	Clioquinol, 3·0
Propaderm	Allen & Hanburys	Beclomethasone dipropionate, 0·025	—
Propaderm-A	Allen & Hanburys	Beclomethasone dipropionate, 0·025	Aureomycin, 3·0
Propaderm-C	Allen & Hanburys	Beclomethasone dipropionate, 0·025	Clioquinol, 3·0
Propaderm-N	Allen & Hanburys	Beclomethasone dipropionate, 0·025	Neomycin sulphate, 0·5
Synalar	ICI	Fluocinolone acetonide, 0·025	—
Synalar-C	ICI	Fluocinolone acetonide, 0·025	Clioquinol, 3·0
Synalar-N	ICI	Fluocinolone acetonide, 0·025	Neomycin sulphate, 0·5
Topilar	Syntex	Fluclorolone acetonide, 0·025	—
Tri-Adcortyl	Squibb	Triamcinolone acetonide, 0·1	Neomycin (as sulphate), 0·25 Gramicidin, 0·025 Nystatin, 100,000 iu/g
Ultradil	Schering	Fluocortolone pivalate, 0·1 Fluocortolone hexanoate, 0·1	—
Ultralanum	Schering	Fluocortolone, 0·25 Fluocortolone hexanoate, 0·25	Clemizole hexachlorophenate, 2·5
Ultralanum Plain	Schering	Fluocortolone, 0·25 Fluocortolone hexanoate, 0·25	—

activity of triamcinolone acetonide in Adcortyl ointment may be compared with those of the same steroid in Ledercort ointment, while the activity of either preparation may be compared with the activity of, e.g. Betnovate ointment.

Occluded test

By employing Betnovate cream it was possible to relate the results for each ointment to those obtained with a 'standard preparation' (Barry & Woodford, 1974). By this means the results for all preparations employed in the three separate ointment trials were comparable with each other and with those obtained previously with the gels and creams.

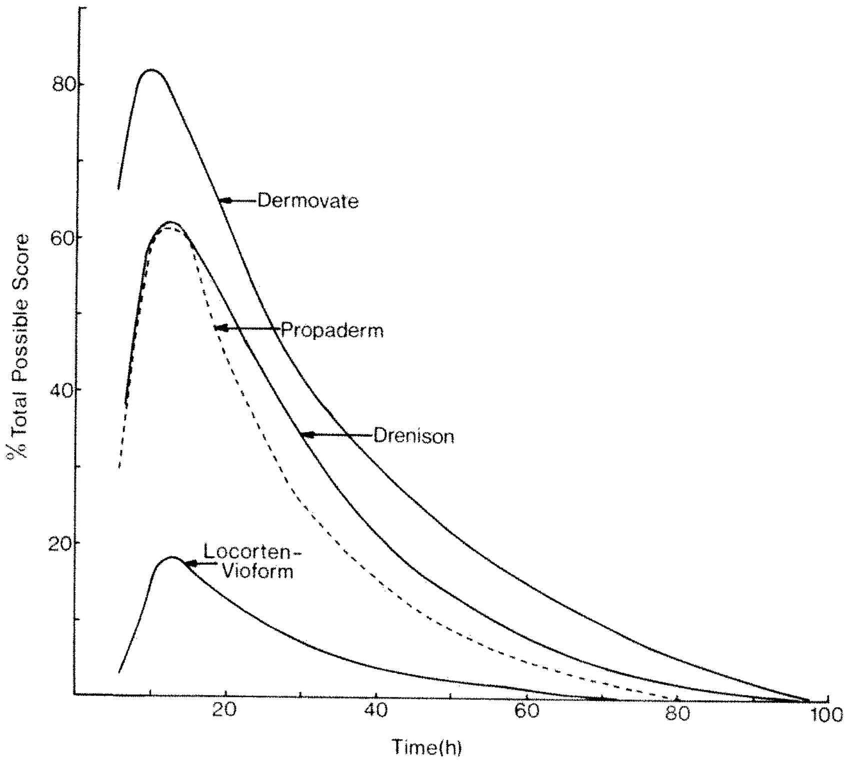

FIGURE 1. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

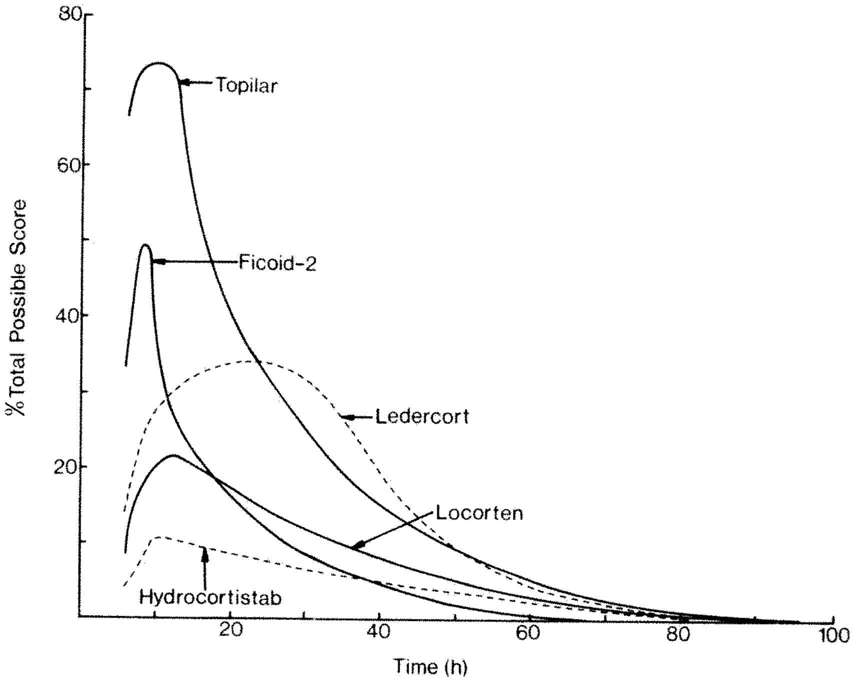

FIGURE 2. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

TABLE 2. Blanching response to thirty-one corticosteroid ointments, arranged in rank order of area under the curve values

Ointment	Blanching response*						
	Area under the curve %×h†		Summed % total possible score‡		Tm/T0 mean value, square root transformation§	% Total possible score on re-occlusion¶	
	Occluded	Non-occluded	Occluded	Non-occluded	Occluded	Occluded	Non-occluded
Synalar-N	2750	2530	397	373	5·57	63	54
Synalar-C	2720	2660	396	374	5·57	63	52
Synalar	2640	2680	400	378	5·60	50	46
Dermovate	2640	—	519	—	6·43	67	—
Betnovate-A	2240	1230	393	323	5·56	19	13
Ultralanum	2190	—	375	—	5·39	19	—
Ultralanum Plain	2170	—	375	—	5·43	19	—
Betnovate-C	2170	1620	376	331	5·45	19	13
Betnovate-N	2140	1630	387	354	5·51	38	25
Betnovate	2080	1870	407	381	5·66	35	25
Drenison	1880	—	348	—	5·23	13	—
Drenison with Neomycin	1800	—	330	—	5·08	13	—
Topilar	1680	1770	429	551	5·84	38	13
Haelan	1570	—	328	—	5·06	13	—

Propaderm	1540	1680	307	345	4·90	19	19
Haelan C	1490	—	294	—	4·80	25	—
Propaderm-A	1470	1270	286	327	4·74	6	19
Propaderm-C	1430	1440	277	320	4·65	13	19
Propaderm-N	1420	1640	286	337	4·73	19	19
Adcortyl	1340	—	175	—	3·63	13	—
Ledercort	1290	—	186	—	3·75	13	—
Tri-Adcortyl	1120	—	167	—	3·58	13	—
Deflamene-A	752	—	177	—	3·63	6	—
Deflamene	724	—	181	—	3·67	6	—
Ultradil	692	—	210	—	4·05	13	—
Locorten	677	416	114	78	3·00	6	6
Ficoid-2	669	—	222	—	4·17	6	—
Ficoid-2 Plus	614	—	209	—	4·04	6	—
Locorten-Vioform	421	311	65·5	86	2·25	6	6
Hydrocortistab	368	—	55·4	—	2·05	0	—
Hydrocortisyl	175	—	43·0	—	1·66	0	—

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* Values quoted to three significant figures for data based on full panel of ten volunteers.

† Obtained by planimetry of the blanching profile.

‡ The percentage of the total possible scores summed for all volunteers over all reading times.

§ The Tm/10 value is the square root transformation of sum of scores (Tm) divided by the number of volunteers (10). The minimum significant range value k = 1·02 ($P < 0{\cdot}05$), i.e. if the Tm/10 values of two preparations differ by more than 1·02 there is a significant difference between those preparations.

¶ Re-occluded for 12 h, 8 days after commencement of experiment: three volunteers (statistical treatment inapplicable).

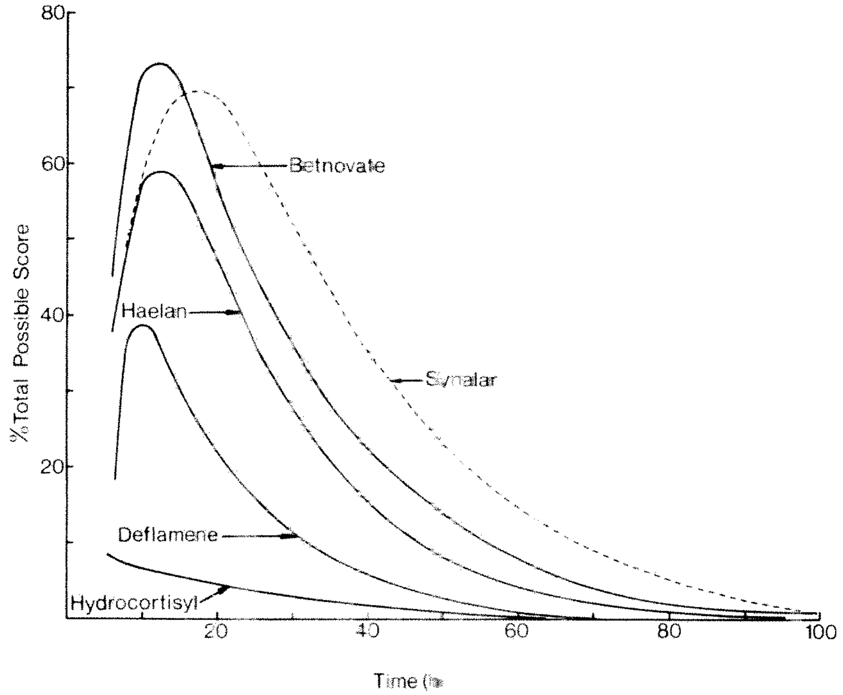

FIGURE 3. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

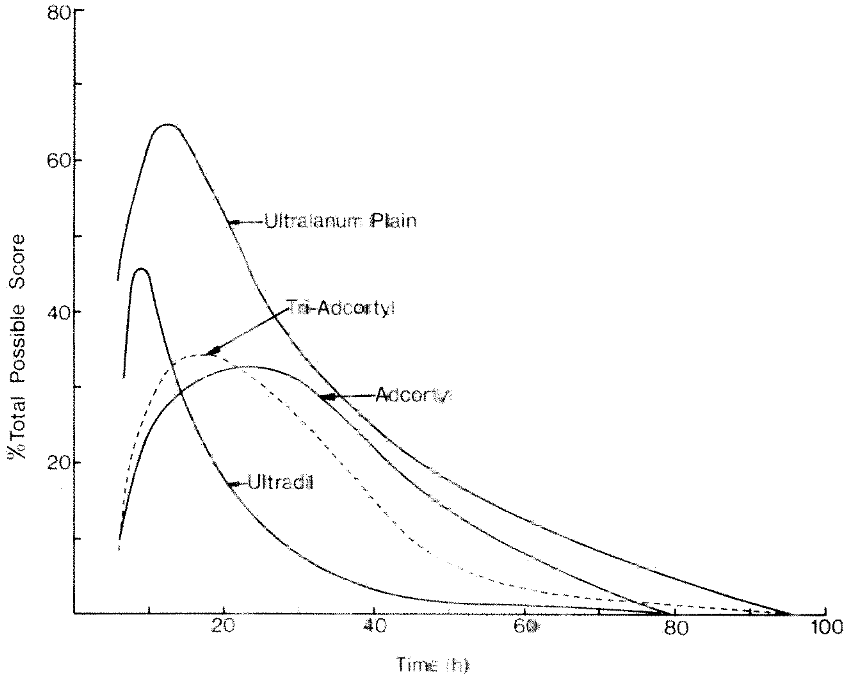

FIGURE 4. Percentage of the total possible score as a function of time for formulations indicated—occluded test.

Blanching profiles for the additive-free ointments are shown in Figs 1–4, separated and occasionally dotted for clarity. The presence of anti-microbial additives had no apparent effect on the shape of the blanching curves, exceptions being Locorten-Vioform and Tri-Adcortyl ointments, and so only the results for the last-mentioned preparations are shown graphically. Experimental points were omitted but all lay on or near the curves as drawn. Many preparations gave curves with a peak in the 9–12 h region with a marked fall-off thereafter. Exceptions were Synalar (where the peak was in the 16–18 h region: Fig. 3), Tri-Adcortyl (where the peak was in the 16–18 h region but the 32 h response was more than 70% of the peak value: Fig. 4), Ledercort and Adcortyl (where the blanching curves tended to plateau over the region 15–30 h with a maximum at 24 h: Figs 2 and 4) and Hydrocortisyl (where the scores decreased continuously after 6 h: Fig. 3). It is interesting to note the similarity of the curves for Adcortyl and Ledercort, together with their difference to the blanching profiles for the other 'plain' formulations (i.e. those not containing antimicrobial additives). It appears that the characteristic curves for Adcortyl and Ledercort were a reflection of the rather poor bio-availability of triamcinolone acetonide from these ointment bases. The summed percentage of the total possible scores (i.e. the percentage of the total possible scores summed for all time periods) for the 'plain' and corresponding additive-containing preparations were within 90% except for Locorten-Vioform which was 58% that of Locorten (Table 2).

The scores for each volunteer were summed over all reading times and analysed statistically as the nontransformed data and as each of five (x^{-1}, $x^{-\frac{1}{2}}$, $\log x$, $x^{\frac{1}{2}}$ and x^2) transformations (Barry & Woodford, 1974). Tests for non-additivity indicated a preference for the square-root transformation and the analysis of variance was calculated using those values. For the three occluded trials treated as one experiment, statistically highly significant differences existed between the preparations ($F = 33·42$, $P<0·01$) and between the volunteers ($F = 14·88$, $P<0·01$). Calculation of the minimum significant range value k using the Studentized range test (Goldstein, 1964) permitted comparison of the proprietary formulations. Significant differences between preparations occurred when their Tm/10 mean values (Table 2) differed by a figure greater than the value of k. For example, the non-significance range for Propaderm was 4·90 ± 1·02, i.e. 5·92–3·88. Hence, Propaderm was not significantly different to the preparations in Table 2 from Synalar-N to Propaderm-N with the exception of Dermovate, which was significantly more active.

Re-occlusion of the application sites in three volunteers (for which, therefore, statistical analysis was inapplicable) permitted estimation of steroid retention in the stratum corneum. The degree of maximal blanching was, in general, proportional to the ranking of preparations (Table 2) with obvious steroid reservoirs being established in the case of Dermovate and the Synalar preparations. Subsequent re-occlusion showed that the presence of steroids in the skin could not be demonstrated after 12 days, with the exception of Dermovate and Synalar which disappeared after 14 days.

Non-occluded test

Betnovate cream was employed as a 'standard preparation' so that the results for the non-occluded ointments could be compared with those obtained with the corresponding creams and gels (Barry & Woodford, 1974).

Blanching profiles obtained are shown in Fig. 5 (note contracted ordinate compared with Figs 1–4). In general, the shapes of the curves obtained in the occluded and non-occluded tests were similar except that Synalar showed a more gradual, almost linear, decrease from the peak value (the 48 h response was about 50% of the 12 h value: Fig. 5). As in the case of the occluded experiments the presence of antimicrobial additives had little apparent effect on the shapes of the blanching curves.

The area under the curve and summed percentage of the total possible score values obtained in the non-occluded test were often of the order of those produced by the occluded procedure (Table 2).

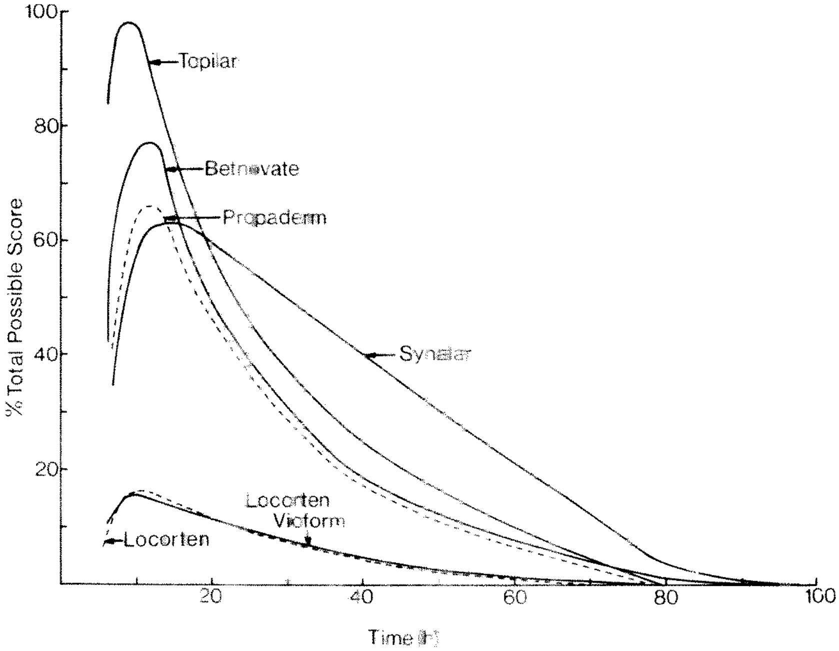

FIGURE 5. Percentage of the total possible score as a function of time for formulations indicated—non-occluded test.

This is a reflection of the occlusive nature of ointment bases: apparently little increase in blanching response was produced by further occluding the ointment sites with polyester film.

Re-occlusion of the non-occluded sites produced very similar results to those of the occluded experiment. This observation is in accord with the occlusive nature of the ointment base, whose capacity to establish a steroid reservoir in the skin is not apparently augmented by Melinex film.

Comparison of ointments and creams

The results obtained with the ointments will now be compared with those produced by the corresponding creams (Barry & Woodford, 1974). Only the more important points which may exemplify general principles will be discussed.

Occluded test

In most cases the shapes of the occluded ointment curves were the same as those of the corresponding creams while the peak heights were often the same or slightly less. Notable exceptions were Adcortyl and Tri-Adcortyl ointments where the peak heights were only 47% and 64%, respectively, of the corresponding creams. The areas under the blanching curves and especially the summed percent total possible scores were much lower for Adcortyl and Tri-Adcortyl ointments than their creams. The vehicle for both ointments is Plastibase: it would appear that at least some corticosteroids have a low thermodynamic activity in Plastibase with a reduced release rate of drug to the skin. Ledercort ointment also produced lower blanching responses than Ledercort cream, implying that triamcinolone acetonide penetrates the skin less readily from proprietary ointments than from creams, irrespective of the proprietary ointment base.

The summed percentage of the total possible scores for the 'plain' ointments and equivalent preparations containing antimicrobial additives were within 90% except for Locorten-Vioform, which was 58% that of Locorten (Barry & Woodford (1974) reported a value of 53% for the corresponding creams). Whether these diminutions in response were due to the presence of clioquinol, the use of different bases or a combination of the two factors remains unknown.

A rank order arrangement of proprietary formulations based on a biopharmaceutical parameter (i.e. area under the curve values) was approximately the same for the ointments and creams. The area under the curve values were often higher in the case of the ointments, especially so in the case of Ultralanum where the ointment was apparently much more active than Ultralanum cream.

With some preparations it was rather difficult to assess the degree of pallor on re-occlusion and because only three volunteers were employed it was difficult to compare accurately the retention of steroid in the skin resulting from ointment and cream application. In many cases the steroid reservoir in the skin 8 days after commencement of the experiment was approximately the same for the ointments and creams. For the Synalar, Topilar and Ultralanum ointments the degree of blanching was 4–5-fold that produced by the creams but the statistical significance of this observation is obscure.

Non-occluded test

The shapes of the non-occluded ointment curves were very similar to those of the corresponding creams with the exception of Synalar where the ointment provided a graph rising to a maximum followed by a fall without the plateau so characteristic of the non-occluded Synalar cream. This may well be a reflection of the occlusive nature of the Synalar ointment base. The percent total possible scores obtained on re-occlusion of the ointments were greater than those produced by the creams, 10-fold increases being apparent for the Synalar preparations: this again was probably due to the occlusive effect of the ointment vehicle.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Mr B.Tolman with the statistical analysis of the results and those manufacturers listed in Table 1 who donated samples.

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Treatment of pemphigus with cyclophosphamide

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Accepted for publication 9 February 1975

SUMMARY

Cyclophosphamide was ineffective in controlling acute disease activity in nine of eleven pemphigus patients, but when given during the remission phase even 50 mg cyclophosphamide per day was sufficient to prevent subsequent relapses in all the patients for quite long periods of follow-up. This small dose of cyclophosphamide could be given to outpatients and there were no side effects.

In an attempt to avoid or minimize the side effects of prolonged corticosteroid therapy in pemphigus, cyclophosphamide has been used in sixteen patients.

MATERIAL AND METHODS

Sixteen patients with pemphigus (pemphigus vulgaris, 13; pemphigus foliaceus, 1; and pemphigus erythematosus, 2) were selected at random. The diagnosis in each case was confirmed by histopathology.

Cyclophosphamide was administered in two dosage schedules: (1) intravenous schedule giving 200 mg cyclophosphamide in 10 ml of distilled water by a slow intravenous injection daily for 20 days, and (2) oral schedule consisting of a 50 mg cyclophosphamide tablet given daily for periods varying from 5 to 13 months. During this period the dosage of corticosteroid was reduced as much as possible and in some cases it was completely stopped. Regular checks were made on the haemoglobin, total and differential leukocyte counts, urinalysis and stools for occult blood in addition to any clinical signs of cyclophosphamide toxicity. The clinical course of the disease in each patient during and after this treatment was compared with the course before this treatment.

RESULTS

Twelve patients were treated with intravenous cyclophosphamide and six patients were given oral cyclophosphamide. Two patients given oral cyclophosphamide had earlier been treated with intravenous cyclophosphamide.

I.V. treated group

Twelve patients were treated with intravenous cyclophosphamide. In one patient, who had pemphigus vulgaris and Hodgkin's disease, cyclophosphamide alone (2 relapses) or with corticosteroids (1 relapse) was able to induce remissions within 1 or 2 weeks. In another patient it was able to control a relapse without the necessity of increasing the dose of corticosteroids. In a third patient treated during two different relapses, cyclophosphamide seemed to induce a remission within 2 weeks during the first relapse, but was apparently without any effect during the subsequent relapse. In one other patient who was treated in the remission phase, the treatment seemed to prolong the remission. In the remaining eight patients treated during the phase of clinical activity, cyclophosphamide did not seem to produce any significant effect on the course of the disease; the disease activity could not be controlled without giving a sizeable dose of corticosteroids, nor were subsequent relapses delayed.

Eight patients tolerated the drug well, but four patients developed side effects. Three patients developed nausea and vomiting, two developed reversible alopecia and one patient each developed fever, leukopenia or diarrhoea. In two patients the drug had to be stopped because of severe side effects.

Oral treated group

All the six patients given oral cyclophosphamide were treated in the remission phase and they used it for 13, 8, 10, 5, 8 and 9 months respectively. These six patients have remained free from clinical lesions for 11, 9, 10, 7, 13 and 29 months respectively ever since they started oral cyclophosphamide and none of them developed any side effects. Salient features in their case histories follow.

CASE REPORTS

Case 1. A 40-year-old female had never been free from pemphigus vulgaris involving the skin and mucous membranes since January 1965 and had throughout been on systemic corticosteroids. In July 1973, while her disease was controlled on 30 mg of prednisone, she was given 50 mg cyclophosphamide orally daily and the prednisone gradually withdrawn during the next 3 months. During the next 11 months of her follow up she has remained completely free from pemphigus lesions.

Case 2. A 50-year-old female had a few lesions of pemphigus vulgaris in January 1972, which healed within 1 month. From April 1972 to August 1972 she had a more severe attack which was controlled with 100 mg of prednisone a day. In November 1972 she had another relapse which was again controlled with 40 mg prednisone a day, but in January 1973 as the corticosteroids were being withdrawn she was put on oral cyclophosphamide in a dose of 50 mg a day. She continued taking cyclophosphamide until September 1973 and was still free from pemphigus lesions when last seen in March 1974.

Case 3. A 50-year-old male had pemphigus of the buccal mucosa in August 1964, followed 2 months later by skin lesions. For nearly 8 years he had lesions off and on, and was on corticosteroids almost throughout this period except for 11 months between November 1970 and October 1971. As he was recovering from his last attack in May 1973, he was given 50 mg cyclophosphamide a day without any corticosteroids and since then he has been almost completely free from pemphigus lesions over a period of 10 months.

Case 4. A 33-year-old female had her first attack of pemphigus vulgaris in December 1972. By March 1973, 75% of her skin had been involved and she was put on 60 mg prednisone a day. As the lesions healed and the corticosteroids were being withdrawn, she was started on 50 mg cyclophosphamide in June 1973 which was continued till December 1973. At follow up in January 1974, she had remained free from lesions (for 7 months) except for two or three lesions which developed in August 1973.

Case 5. A 55-year-old male had his first attack of pemphigus vulgaris in October 1972 which was controlled with 30 mg prednisone a day. After a remission of nearly 1 month, in April 1973, he had a much more severe attack which was controlled with 60 mg prednisone a day. In May 1973 as the lesions were healing, he was given 50 mg cyclophosphamide a day orally and prednisone was gradually withdrawn. Cyclophosphamide was also stopped in January 1974 and he had remained free from lesions for 13 months when last seen in October 1974.

Case 6. A 15-year-old male had three successive attacks of pemphigus foliaceus in May 1971, October 1971 and December 1971, without a complete remission in between. The first two attacks were controlled with 50 mg prednisone a day and the third attack with 60 mg prednisone a day. During the third attack he was also given 200 mg cyclophosphamide daily intravenously for 20 days and this was followed by a complete remission for nearly 1 month. In April 1972, there was a mild recurrence and the patient received 50 mg cyclophosphamide orally per day for the next 9 months. For 29 months, when last seen in October 1974, he had remained free from lesions except for 2 mild attacks, one between July and September 1972 and the other between June and July 1973. Both these attacks were controlled with 20 and 40 mg of prednisone per day.

DISCUSSION

Treatment of pemphigus with corticosteroids in large doses over prolonged periods leads to several side effects some of which are as serious and fatal as the disease itself. With the general acceptance that pemphigus is an auto-immune disease (Beutner, Chorzelski & Jordon, 1970), use of immunosuppressive agents in the treatment of this disease seemed quite logical. Lever & Goldberg (1969) were the first to report successful use of methotrexate in pemphigus and this was followed by several other reports on the use of methotrexate (Lever & Hashimoto, 1969; Jablonska, Chorzelski & Blaszycyk, 1970; Peck *et al.*, 1971; Ryan, 1971), azathioprine (Jablonska *et al.*, 1970; Burton *et al.*, 1970; Krakowski, Covo & Rozanski, 1969; Roenigk & Deodhar, 1973) and cyclophosphamide (Ebringer & Mackay, 1969; McKelvey & Hasegawa, 1971; Krain, Landau & Newcomer, 1972). Methotrexate, however, has gone into disrepute because in some patients it has produced irreversible and serious damage to the liver, while experience with azathioprine and cyclophosphamide is still scanty. We first used cyclophosphamide in a patient having Hodgkin's disease who had also developed pemphigus vulgaris. On three different occasions we could induce remissions in the patient with cyclophosphamide used alone or in combination with corticosteroids (Sood & Pasricha, 1974). In most of our other patients, however, cyclophosphamide seemed to be of no use when given during the active phase of the disease. This corroborates the experience of earlier workers that cyclophosphamide cannot control severe relapses.

In contrast to this, when started during the remission phase, cyclophosphamide was able to prevent recurrence of any significant disease activity for long periods. This small dose of 50 mg a day did not cause side effects in these patients and could be given without admitting the patient to hospital. Complete withdrawal of corticosteroids during this period reduced and reversed the side effects of prolonged corticosteroid therapy and all patients felt very comfortable. It is recommended that pemphigus patients, during the phase of clinical activity, should be treated with corticosteroids and once the remission sets in, corticosteroids should be completely withdrawn and they should be maintained on oral cyclophosphamide. If there are any recurrences, the disease activity should again be controlled with corticosteroids and once the remission sets in, the patient should again be put on cyclophosphamide and the corticosteroids withdrawn completely.

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A follow-up of tetracycline-treated rosacea

WITH SPECIAL REFERENCE TO ROSACEA KERATITIS

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Accepted for publication 10 February 1975

SUMMARY

Seventy patients with rosacea were treated with systemic tetracycline for 6 months. Sixty-eight of them cleared with treatment. After withdrawal of the drug seventeen relapsed immediately and the overall relapse rate over 4 years was 69%. The serum tetracycline levels were not significantly different in two patients who failed to respond. Six patients had rosacea keratitis and responded dramatically within 1 month. Symptoms recurred as the drug was withdrawn. It is suggested that rosacea patients with keratitis should receive early and prolonged tetracycline medication.

Rosacea is a common condition in Britain, yet there are no published data on the natural history of rosacea. Clinical impressions suggest that the disease lasts for many years with relapses and remissions. Sneddon (1966) first reported the beneficial effect of systemic tetracycline on rosacea. Marks & Ellis (1971) later made an objective study of this treatment and since then it has gained universal acceptance. The present study was initiated in order to observe the long term results of tetracycline treatment of rosacea.

METHOD

Seventy-five consecutive patients with acne rosacea were started on oral tetracycline medication and observed at 2-monthly intervals. They were given tetracycline 250 mg twice daily taken 1 hour before meals for 6 months. After this period the tetracycline was gradually stopped over the next month. Clearing of the rosacea was said to have occurred if there was disappearance of the papules and pustules. Residual erythema was not considered to be active disease.

Two of the seventy-five patients were later found to have other diseases, one having discoid lupus erythematosus and one acne vulgaris. Three patients failed to attend for follow up. The remaining seventy patients (50 female and 20 male, Table 1) were seen regularly at 2 month intervals for from 1·5 to 4·5 years. The average period of follow-up was 2·6 years.

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Tetracycline serum levels

Random tetracycline serum levels were determined by one of us (A.G.K.). The method was based on the extraction of tetracycline complexes and the measurement of fluorescent intensity with a fluorometer (Kohn, 1961). The specimens were taken 1–3 h after the morning tablet. Each patient had at least one serum tetracycline estimation. The two patients who failed to improve with tetracycline therapy had three separate estimations.

Ocular symptoms

No special study was made of eye symptoms and signs in rosacea. However six female patients were found to have keratitis; all were seen by an ophthalmologist.

RESULTS

At the end of the initial 6 months of tetracycline therapy only two patients (1 male and 1 female) still had active rosacea (Fig. 1). However seventeen of the seventy patients relapsed during the month of tetracycline withdrawal. During the period of observation forty-four patients suffered one relapse and twenty-six two or more relapses. With each relapse a further course of tetracycline 250 mg twice daily was given for 3 months. At the end of the follow-up twenty-one patients were still requiring

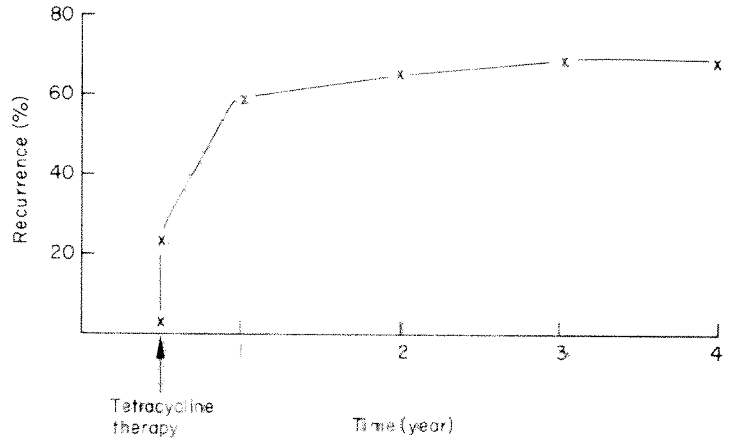

FIGURE 1. Percentage recurrence of rosacea after systemic tetracycline.

TABLE 1

	Male	Female
Average age	53	48
Keratitis	—	6
Rhinophyma	4	—
No response to tetracycline	1	1
Response to tetracycline	19	49

Total patients 70.

treatment. There were twenty-six patients observed for periods in excess of 3 years. Of these, fifteen still had active rosacea.

Tetracycline serum levels

There was no statistical difference between the levels in those who improved and those who did not (Table 2).

Ocular symptoms

Six patients, all female, had rosacea and keratitis. Three had the keratitis diagnosed before entering the study but were not on oral tetracycline. One of these had a unilateral hypopyon and was blind in that eye. The other three had eye symptoms on their first visit; keratitis was diagnosed and confirmed. The keratitis in all six patients was suppressed within 1 month of tetracycline therapy. It recurred in all patients within 3 months of stopping the treatment, and responded as previously to a further course. Thereafter they were maintained on a small dose of tetracycline.

TABLE 2. Tetracycline serum levels

		Levels μg/ml	
	Determinations	Range	Average
No response to tetracycline	6	1·0–2·1	1·5
Response to tetracycline	73	0·5–3·0	1·4

DISCUSSION

The present study, although not controlled and double-blind, does confirm Sneddon's (1966) observations on the efficacy of tetracycline medication for rosacea. Sixty-eight (97%) of the patients cleared with treatment. However seventeen (24%) relapsed within a few days of stopping the drug and forty-two (60%) within a further 6 months. The overall relapse rate over 4 years was 69% (Fig. 1). Thus tetracycline is purely suppressive in rosacea. This conclusion was also reached by Sneddon (1966) from the results of a postal survey following his clinical trial. Wereide (1969) observed his patients for the duration of tetracycline therapy and for a short time after stopping the drug. He noted a 78% immediate relapse rate of the rosacea. He did comment, however, that in many cases a small dose of tetracycline (250 mg on alternate days) will control the disease.

A few patients do not respond to the usual dosage of tetracycline. The two such patients in the present study had tetracycline serum levels within the range normally expected (Table 2). Neither patient was given increased doses of tetracycline.

The incidence of keratitis in association with rosacea has varied between publications. Goldsmith (1953) felt that conjunctivitis and blepharitis were common in rosacea but that keratitis was rare. Borrie (1953) found keratitis to be the commonest ocular lesion associated with rosacea, occurring in 3% of patients. Starr & Macdonald (1969) described the detailed eye examination of fifty-seven consecutive rosacea patients seen at a London Skin Department. 58 % had some eye disease and 33% had involvement of the cornea. There was an equal sex incidence. Sneddon (1966) noted prompt clearing of both the rosacea and the keratitis with tetracycline, in all four of his patients. Marmion (1969) noted subjective and objective improvement in the keratitis with systemic tetracycline therapy.

The six patients in the present study all cleared up within 1 month of treatment but relapsed within 3 months of stopping the tetracycline. The rosacea and keratitis did seem to respond equally. The patients were thereafter maintained on a small dose of the drug and remained free of active disease. Permanent scarring and hypopyon formation are severe but rare end products of keratitis. Thus rosacea patients with eye symptoms should be adequately examined and maintained on long-term tetracycline with a regular follow-up. The potentially disastrous results of rosacea keratitis may be prevented by early and prolonged tetracycline treatment.

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British Journal of Dermatology (1975) **93**, 581.

Case Reports

Hodgkin's disease in a patient with follicular and dermal mucinosis and spontaneous ooze of mucin (mucinorrhoea)

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Accepted for publication 7 February 1975

SUMMARY

A patient with follicular and dermal mucinosis is described. Although the skin histology was of a benign nature the patient died from an associated underlying reticulosis. Spontaneous mucinorrhoea subsided with therapy for Hodgkin's disease.

Pinkus (1957) used the term alopecia mucinosa to describe a disease which presents clinically as sharply defined plaques and histologically as follicular degeneration leading to alopecia and characterized by oedema in the outer root sheath and sebaceous gland. Jablonska, Chorzelski & Lancucki (1959) reported further cases in which hair loss was not apparent and proposed the alternative name follicular mucinosis. In 1964 Okun & Kay described three patients who, in addition to the characteristic histological changes, showed an unusual feature of mucinous degeneration of the dermis. Braun-Falco (1957) recognized two forms of the disease, a benign type and a symptomatic group associated with an underlying reticulosis. The patient to be described presented with follicular and dermal mucinosis with mucinorrhoea as manifestations of Hodgkin's disease.

CASE REPORT

A man of 50 years presented in January 1973 with ulcers on the right side of the nose and under the right eye for 6 months. He also complained of a swelling in the right groin increasing in size for 3 months. A rash on the back, forehead and right thigh had not changed over the past 6 months. Apart from loss of half a stone in weight over the past 2 months his general health was good. There was no relevant past or family history. On examination there were ulcerated nodules on the face and indurated plaques on the forehead. There were extensive plaques, nodules and follicular lesions on the back (Fig. 1). On the chest were follicular lesions, some of which had aggregated together to form plaques, lesions resembling lichen spinulosus, and nodules which appeared yellow-brown on diascopy (Pinkus, 1957). On the right thigh there was a large indurated plaque from the whole surface of which oozed

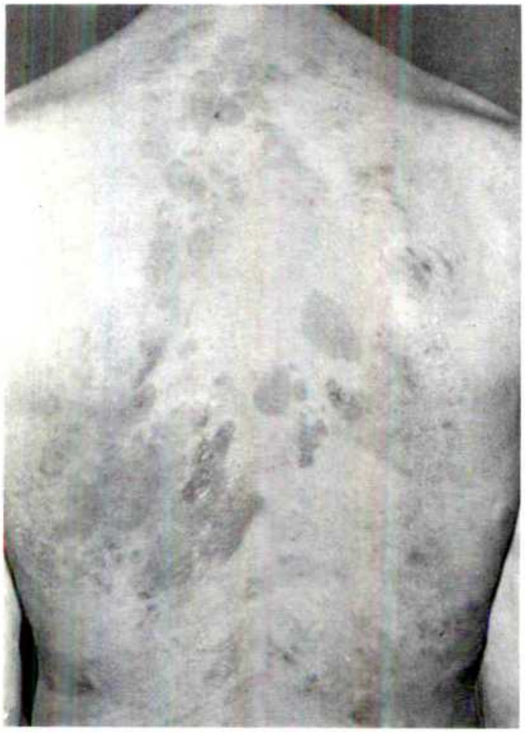

FIGURE 1. Extensive plaques, nodules and follicular lesions on the back.

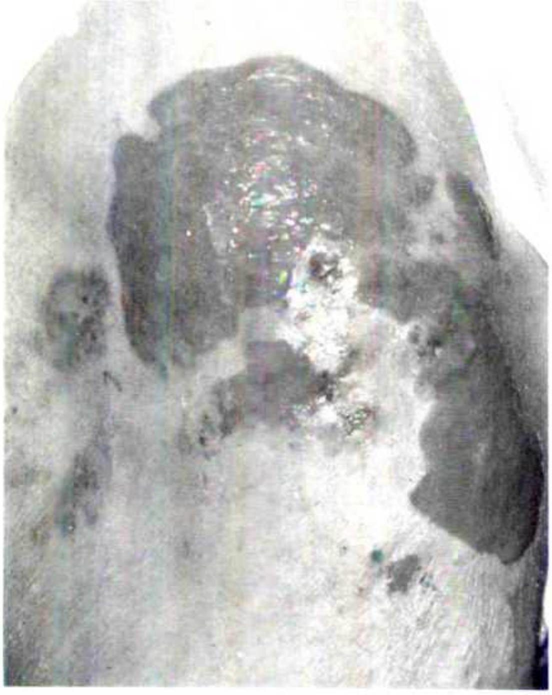

FIGURE 2. Mucinous ooze from plaque on right thigh before treatment.

a clear mucinous fluid (Fig. 2). The ooze was accentuated by squeezing the plaque. The right inguinal lymph nodes were enlarged, firm, matted together and non-tender. The liver was enlarged three finger breadths below the costal margin and of normal consistency and the spleen was palpable on inspiration. Many investigations were carried out, of which the following are pertinent: Hb 12·8 g%; WBC 12,900/cmm, neutrophils 88%, eosinophils 2%, basophils 0%, lymphocytes 4%, monocytes 6%; serum Ca 4·3, 4·4, 4·6 mmol/L; serum phosphate 2·1, 2·3, 2·4 mmol/L; serum folate 2·8 Mμg/ml (4–18); serum B_{12} 330 μμg/ml (160–900); 3 day stool, wt 420 g, fat 12·5 g/sample; 5 h urine, vol 270 ml, D-xylose 3·0 g/sample; barium meal, non-specific malabsorption pattern; serum bilirubin 0·3 mg%; alkaline phosphatase 26, 28 KA units; serum transaminases normal; liver scan, count density patchy possibly due to minute small metastatic deposits; serum iron 25 μg%, T.I.B.C. 180 μg%, 14% saturation; bone marrow, non-specific marrow overactivity ('irritation'); serum albumin 2·4 g%, serum globulins, α_1 1·6 g%, α_2 0·9 g%, β 0·9 g%, γ 1·8 g%, total protein 6·8 g%; IgG 2288 mg% (700–1800), IgA 260 mg% (90–450), IgM 100 mg% (60–280); X-ray chest, nodularity of the parenchyma of both lungs; lung function tests, transfer factor diminished, hypoxaemia with normocapnia, in keeping with a lesion at alveolar level; skeletal survey, generalized thinning of the bony texture.

The fluid which exuded from the plaque on the right thigh stained positively with Alcian blue and metachromatically with toluidine blue demonstrating the presence of mucin.

A biopsy was taken from the weeping plaque and the tissue was fixed in formalin, processed and stained with haematoxylin and eosin. Examination revealed an extensive patchy infiltrate consisting of histiocytes with many eosinophils (Fig. 3). There were no atypical forms and mitoses were infrequent. Some hair follicles showed marked damage and contained keratinous material with occasional

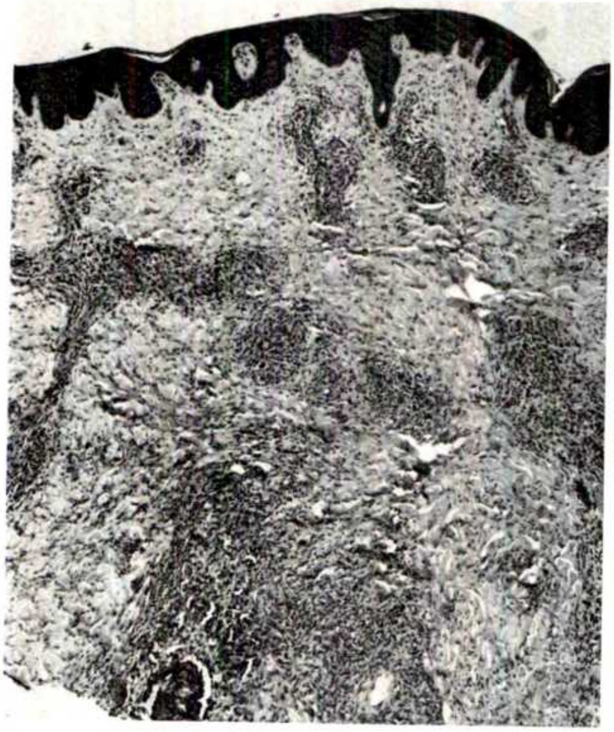

FIGURE 3. Histological features of weeping plaque (H & E, × 50).

foreign body giant cells. Special stains with Alcian blue revealed mucinous change affecting occasional hair follicles and the dermis where it was restricted to sites occupied by the cellular infiltrate. The histological changes were in keeping with follicular and dermal mucinosis with no evidence of a reticulosis.

Biopsy of a lymph node from the right inguinal fossa was fixed in formalin, processed and stained with haematoxylin and eosin. The histological findings were as follows: 'There is destruction of the normal architecture with several foci of necrosis. The capsule is infiltrated with plasma cells and there is an increase in the number of eosinophils. Many pleomorphic reticulum cells are present, some of which show increased mitosis with occasional 'mirror-image' cells. These histological features are thought to represent a malignant reticulosis, most probably Hodgkin's disease'.

In summary, the investigations confirmed the clinical impression of a malignant reticulosis of the inguinal lymph nodes and probably affecting several internal organs. The clinical appearance of the skin favoured a diagnosis of follicular mucinosis secondary to cutaneous reticulosis whereas histological examination of the skin showed no evidence of malignancy. As there was a strong suspicion of cutaneous reticulosis two further skin biopsies were carried out but both showed pathological features similar to those seen in the initial biopsy.

Treatment with quadruple chemotherapy, mustine hydrochloride, vinblastine, procarbazine and prednisone, was instituted with help from a haematologist. Two weeks later there was marked improvement in all the skin lesions (Fig. 4). The indurated plaque on the right thigh largely resolved and the mucinous ooze subsided. A biopsy of the plaque showed a reduction in the dermal mucinosis and

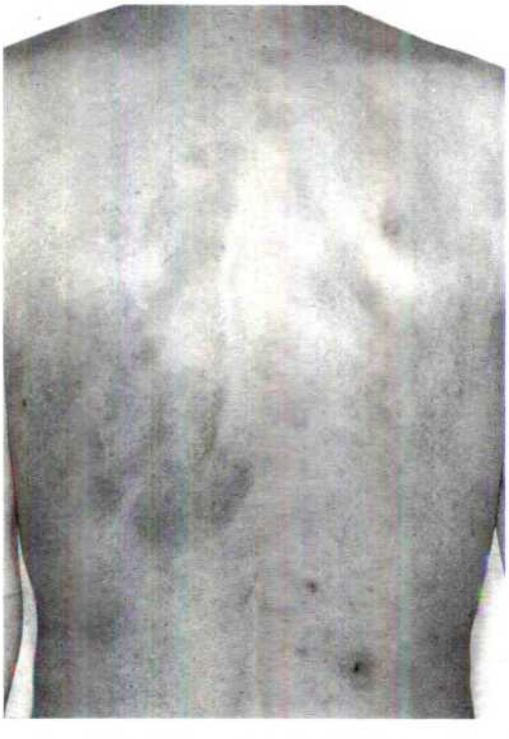

FIGURE 4. Appearance of back after treatment for Hodgkin's disease.

cellular infiltrate. There was also great systemic improvement, the liver and spleen were not palpable and the radiological changes in the lungs were less obvious. This improvement was maintained for 3 months with repeated courses of treatment when there was a recurrence of mucinous ooze from the plaque on the right thigh which became more obvious, fresh skin lesions developed and the patient's general health began to deteriorate. Despite further chemotherapy, including bleomycin and supportive treatment, the patient pursued a downhill course and succumbed to his illness in August 1973. A post mortem showed that the lymph nodes, liver, spleen and lungs were infiltrated with pleomorphic reticulum cells with a high level of mitotic activity. Binucleate forms of Sternberg-Reed cells, giant cells, plasma cells and occasional eosinophils were noted. The patient was considered to have died from Hodgkin's disease Grade IV. Careful search failed to reveal any evidence of malignant change affecting the skin.

DISCUSSION

In the patient described, the clinical features of follicular mucinosis were confirmed by histology. The clinical appearance and widespread distribution of the nodules and plaques were very suggestive of cutaneous reticulosis especially as the patient was known to have systemic involvement. However careful examination of biopsies from four suspicious skin lesions failed to reveal any conclusive histological evidence of malignancy.

Emmerson (1969) reviewed forty-seven patients suffering from follicular mucinosis collected over 16 years from 1952 to 1968. He compared the histological changes in the skin with the natural history of the disease and found that in eight patients (17% of the total) reticulosis was recognizable early in the illness. In all patients with an associated reticulosis the skin infiltrate showed cellular atypicality with increased mitotic activity. In contrast, in those patients in whom the disease followed a benign course, the infiltrate, no matter how dense, consisted of benign inflammatory cells of which the majority were lymphocytes with a variable number of pale staining histiocytes and eosinophils. In no case was there a transition from follicular mucinosis to reticulosis.

In view of Emmerson's (1969) findings a representative histological section of a skin lesion from the patient was sent to Professor J.A.Milne, Department of Dermatology, Glasgow, who confirmed the benign nature of the infiltrate. This patient presented with follicular mucinosis with no histological evidence of cutaneous malignancy but died from an associated systemic reticulosis. The great increase in the number of eosinophils in the skin infiltrate may have prognostic significance.

The ability to express mucin from follicular openings by squeezing skin lesions—induced mucinorrhoea—is said to be pathognomonic in doubtful cases of follicular mucinosis (Haber, 1961). Apart from a comment by Witten (1962) on a patient with follicular mucinosis, there has been no previous report of spontaneous mucinorrhoea in this disease. Resolution of the ooze following therapy for Hodgkin's disease suggests that spontaneous mucinorrhoea was a secondary manifestation of reticulosis.

The other unusual feature in this patient was the presence of mucin in the dermis, a feature which has been reported only once before (Okun & Kay, 1964). Both the mucin and the cellular infiltrate were restricted to the same sites and responded favourably to treatment for Hodgkin's disease, suggesting a causal relationship. It is likely that induced follicular mucinorrhoea (Haber, 1961) is produced when skin which is the site of follicular mucinosis is subjected to squeezing. The origin of spontaneous mucinorrhoea is less certain but may be due to a 'leak' from a dermis which has undergone mucinous degeneration, either in response to an underlying lymphoma or as a reaction to the dermal cellular infiltrate.

British Journal of Dermatology (1975) **93**, 587.

Leprechaunism

CASE REPORT IN A BLACK AFRICAN CHILD

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Accepted for publication 28 February 1975

SUMMARY

A case of leprechaunism is described. This is thought to be the first reported in a black child.

Leprechaunism is an exceedingly rare disorder. The first formal record was reported by Donohue in 1948 under the name of 'Dysendocrinism'. Less than ten cases have since been described (Donohue & Uchida, 1954; Evans, 1955; Patterson & Watkins, 1962; Salmon & Webb, 1963; Kallo, Lakatos & Szijarto, 1965).

The syndrome is poorly defined, its cause unknown, and survival beyond early childhood uncommon. Salmon & Webb (1963) suggested a tetrad of primary features:

Characteristic facies.

Dystrophy with dwarfism, marasmus, and bony abnormalities

Defective immune response with atrophy of lymphoid tissue, cystic degeneration of thymus, and liability to infections.

Defects of carbohydrate metabolism and endocrine systems.

Various associated developmental abnormalities have been recorded (Table 1). Their significance is not clear.

CASE REPORT

A black infant girl, aged 2 months, was referred to the Paediatric Department of this hospital. Pregnancy was uneventful and terminated at 38 weeks. Consanguinity was denied, and there was no history of drug administration during pregnancy. The infant was an only child and no other congenital abnormalities were reported in the family.

The birth weight was not recorded, but at the age of 2 months the child weighed 2·5 kg. She failed to thrive and despite an adequate diet her weight dropped to 2·3 kg at 3 months of age. She suffered from recurrent bouts of bronchopneumonia and succumbed at 4 months of age to a viral infection of

TABLE 1. Features of Leprechaunism

Summary of six other cases (Salmon & Webb, 1963)			Our case
Sex	4	F	F
Gestation	3	Short	Normal
Consanguinity	3	+	—
Facies	6		
Widely spaced eyes		+	+
Prominent eyes		+	+
Large low set ears		+	+
Loose skin		+	+
Excessive lanugo hair		+	—
Marasmus	5	+	+
Dwarfism	6	+	+
Retarded bone age	6	—	—
Alkaline phosphatase	2	Low	Lower limit of normal
Muscle mass	5	Wasted	+
Breasts	5	Enlarged	Normal
Genitalia	5	Enlarged	Hypertrophy
Urine ketosteroids	2	Normal	Not tested
Liability to infection	3	+	+
Cystic thymus	3	+	—
Lymphoid hypoplasia	3	+	—
Fasting blood sugar	3	Low	Low
Serum insulin		Not tested	High
Insulin response	2	Prolonged	Not tested
Islet hyperplasia	3	+	—
Liver glycogen	3	+	Not tested
Kidneys	3	Tub. dilat.	—
Aminoaciduria	2	—	—
Life span	3	Less than 1 year	Short

The summary shows the number of cases with positive findings (+ positive; – negative). The other cases were either not investigated or the features were not present.

the lungs. The face showed a wizened, old man appearance; the head was disproportionately large, the chin pointed, the ears low set and the eyes prominent and widely spaced (Fig. 1). The skin hung in folds on a wasted frame and showed a remarkable hyperelasticity and thickness, especially on the face and extremities (Fig. 2). The skin of the trunk was, by contrast, rather thin. The subcutaneous tissue was largely absent and the muscle mass grossly wasted. The hands and feet were large. The breasts were normal, but the clitoris was moderately enlarged. The cry was low and husky.

Investigations

The haemoglobin was 11·3 g%; white cell count 11,200; neutrophils 50%; lymphocytes 44%; monocytes 6%. Blood urea was 26 mg%; serum potassium 5·9; sodium 127; chloride 102. Serum protein was 6·8%; albumin 3·4; globulin (alpha 1 0·5; alpha 2 0·9; beta 1·0; gamma 1·0). Serum alkaline phosphatase 6 King-Armstrong unit (lower limit). Urine analysis normal. No aminoaciduria. X-rays of the bones showed no abnormalities and the bone age was normal. Tests of carbohydrate metabolism: a low fasting blood sugar was found on two occasions. The child had two episodes of

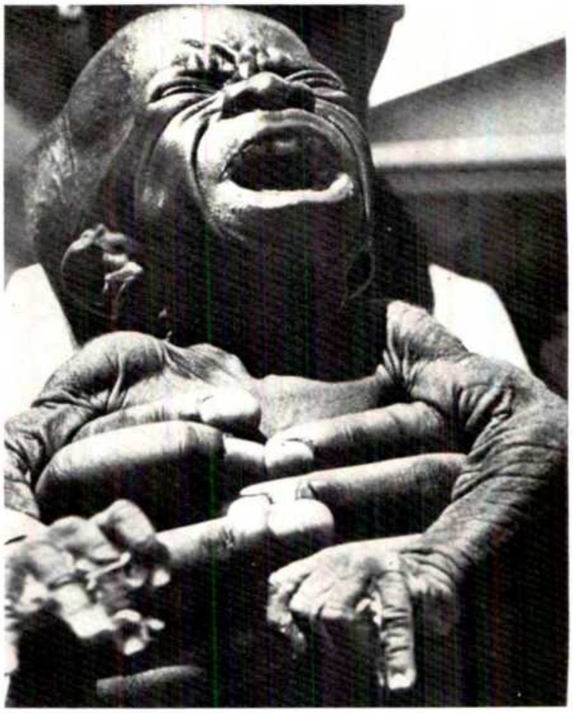

FIGURE 1. Leprechaunism. Typical wizened old man appearance.

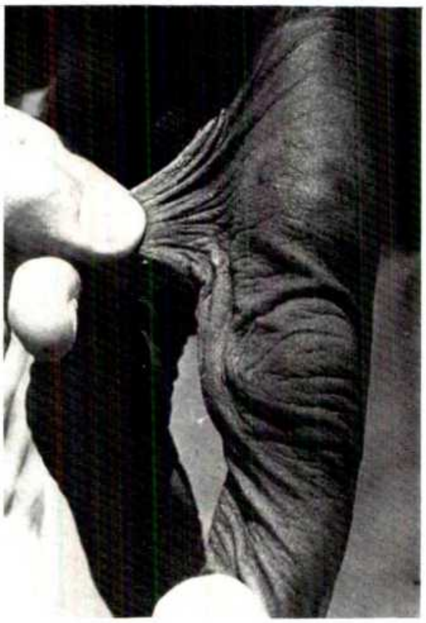

FIGURE 2. Leprechaunism. Hyperelasticity of the skin.

hypoglycaemic coma with blood sugar values of less than 30 mg%. A full glucose tolerance curve was not performed, but after a fast of 4 h the patient showed a blood glucose of 35 mg% and a serum insulin of 40 I.U.

Skin biopsy: The epidermis showed mild hyperkeratosis with a basket-weave appearance of the horny layer, and mild liquefaction degeneration of the basal layer. The dermis showed a mild perivascular lymphocytic infiltrate. Collagen and elastic tissues were normal, except for a minor decrease of the elastic tissue in the sub-papillary dermis.

Post mortem examination

This confirmed the clinical features. The cause of death was found to be an interstitial pneumonia involving most of the left lung and the lower lobe of the right lung. No abnormality was detected in the reticulo-endothelial system, except that large amounts of haemosiderin were deposited in the liver and spleen (Smith, 1970). The pancreatic islet cells were normal. All other organs were found to be normal both macroscopically and histologically.

DISCUSSION

Very little is known about the syndrome of leprechaunism, partly because of the small number of cases so far described and partly because of variations in assessment of the cases.

The early cases reported by Donohue & Uchida (1954) were extensively studied only at post mortem, whereas Evans (1955) described pathological changes during life. More detailed investigations were carried out by Patterson & Watkins (1962) who showed that the clinical picture changes with age, and by Salmon & Webb (1963). The aetiology and pathogenesis of leprechaunism are poorly understood and it is possible that the term embraces more than one condition. The inheritance is probably recessive but no genetic studies to support this view have been performed. Donohue & Uchida's (1954) and Kallo's *et al.* (1965) cases were sisters.

The clinical features of our case closely resemble those reported in the literature. The abnormalities are summarized in Table 1. Carbohydrate metabolism was found to be defective. The presence of an increased serum insulin and a low fasting blood sugar supports the view that the abnormality is related to hyperinsulinism (although the histology of the pancreatic islet cells was normal). This relationship is not found in infants affected by nutritional marasmus where the low blood glucose is associated with hypoinsulinism (James & Coore, 1970). In one reported case of leprechaunism diabetes mellitus developed later. This manifestation was probably due to exhaustion of the islet cells of the pancreas. Assays of sex-hormones were not performed in our patient, but there was evidence of clitoral hypertrophy. The X-ray investigation of bones failed to show delayed ossification or other abnormalities; these changes are usually progressive (Salmon & Webb, 1963), and it is possible that the child died before their development.

Leprechaunism may simulate cutis laxa or the Ehlers-Danlos syndrome. In cutis laxa, however, the skin is thin and atrophic and after stretching returns slowly to its normal position. Histologically elastic tissue is decreased in amount and shows marked fragmentation. These features are distinctly different from those observed in our infant in whom the skin was hyperelastic, but thickened and returned readily to its normal position once the deforming force was removed. The Ehlers-Danlos syndrome usually presents with a positive family history. It shows hyperextensibility of the skin, and other associated features—hyperextensibility of joints, easy scarring and tissue paper scars, musculo-skeletal abnormalities, easy bruising. These were not seen in our infant. The abnormalities in carbohydrate metabolism and in the external genitalia and the peculiar elfin face are not characteristic of the

Ehlers-Danlos syndrome. A number of other conditions may show hyperelasticity and a corrugated appearance of the skin (pseudoxanthoma elasticum, anetoderma, cutis verticis gyrata) but do not in any way resemble leprechaunism.

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British Journal of Dermatology (1975) **93**, 593.

Multiple keratoacanthomas: association with deficient cell mediated immunity

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Accepted for publication 28 February 1975

SUMMARY

A case of multiple keratoacanthomas associated with deficient cell mediated immunity in a 44-year-old male Caucasian is presented. Many attempts to restore the lymphocyte function (transfer factor, levamisole) have failed but the treatments may have had a curative effect on the development of new lesions. The significance of impaired cellular immune defense mechanisms is discussed.

CASE REPORT

A 44-year-old male Caucasian was seen in July 1970 with a 3 years' history of multiple asymptomatic nodules, 5 mm–3 cm in diameter. These had a central keratinous crater, and were located on the backs of the hands, forearms, ears and face. Also present in these areas were whitish atrophic scars. The clinical and histological diagnoses were compatible with keratoacanthoma. No history of exposure to carcinogens, tar-derived products or excessive sunlight was found. The patient received no treatment. Re-examination in March 1971 showed a complete involution of the tumours. However on the left nostril one of them, which grew as an enormous inflammatory tumour and was histologically confirmed, involuted to leave a large mutilating and perforating scar. Three months later, new lesions appeared on the fingers, the upper lip and the right nostril.

Some lesions were removed surgically but most of them healed spontaneously within 2 months.

The patient did well without any treatment until October 1973, when four new lesions occurred on the forehead and the cheek.

There were no signs of infection, anaemia or loss of weight. Physical examination was normal. Sedimentation rate, blood urea, serum iron, and blood sugar concentrations were within normal limits; WBC : 4300 / mm^3. ENT examination and chest X-rays were normal. The patient had no relevant past medical history and was not taking any drug or medicine. A search for occult malignancy was negative.

Immunological findings

The immunoglobulin level was normal. Isohaemagglutinins were present and there was a high titre of specific antibody after antitetanus and antipolio immunization. Immuno-electrophoresis showed an absence of M component.

There was an absence of delayed reactions to repeated intradermal injection of tuberculin (50 units) and candidin (1/100). Dinitrofluorobenzene, (DNFB) 2000/γ, in equal volumes of oil and acetone was applied to an area approximately 20 mm in diameter on the medial aspect of the forearm not used for immunization. The area was occluded with gauze and plastic tape for 48 h. Reactions were read 24, 48 and 72 h after the application of DNFB. Erythema and vesiculation at 48 h, usually accepted as evidence of sensitization, were absent after three attempts.

The lymphocyte blood count was $530/\text{mm}^3$. *Lymphocyte transformation*: lymphocyte suspensions were prepared from 80 ml of heparinized venous blood after passage through a Ficoll–Hypaque gradient. 10^6 lymphocytes were cultured with each of the following mitogens for 5 days : 0·025 ml of phytohaemagglutinin P (Difco); 0·5 ml of pokeweed mitogen (Grand Island Biological Company); 250 units of purified protein derivative (Parke-Davis); and 100 protein mitogen units of *Candida Albicans* per ml of medium. The blastogenic response was measured by tritiated thymidine incorporation and recorded as counts per $\text{ml}/10^6$ lymphocytes. Our control patients have an average response ranging from 1·5 to $2\cdot10^4$ cpm. The stimulation levels obtained in our patient with the different mitogens were between 5 and $8\cdot10^3$ cpm.

Two homografts were rejected in 11 days and 17 days.

There was no major structural alteration of a lymph node biopsied in the groin area.

In 1971 the tuberculin test became temporarily positive after BCG. In 1973, repeated BCG scarifications (37·5 mg per week during a 2-month period) failed to confer a sustained specific immunity. It is now permitted to treat certain immunosuppressed patients with BCG. Minor complications have been observed at vaccination sites or in the lymph nodes but not in our case (Bast *et al.*, 1974). Transfer factor, given in four subcutaneous injections of dialysable extracts from 6×10^9 leukocytes from a pool of PPD positive donors at 15 day intervals, was unable to restore the impaired immunological functions. Levamisole therapy (150 mg a day, 3 days a week for 1 month), which is supposed to enhance cell mediated immunity (Churchill & David, 1973), also failed to improve the *in vivo* and *in vitro* immune reactions to various antigens. However, the patient has since been cleared of keratoacanthomas for 10 months, until now.

DISCUSSION

Since the first description by Hutchinson (1889), keratoacanthoma has become a well known entity. It occurs mainly on sun-exposed areas, is usually solitary but less frequently may be multiple (Graciansky & Leclercq, 1961) and rarely occurs as an eruptive and disseminated tumour (Smith, 1948). The lesion heals spontaneously within 6 to 8 weeks and usually does not recur. Keratoacanthoma represents a benign tumour with a pseudo-malignant histological appearance, masquerading as a squamous cell carcinoma which has to be considered from the clinical appearance (Lapiere, 1961). The causative agent is still unknown.

Recurring solitary keratoacanthomas have been reported by many authors (Rook, Kerdel-Vegas & Young, 1967; Degos, Cottenot & Civatte, 1958; Stevanovic, 1969), but we were unable to find reports of multiple keratoacanthomas associated with a deficit of cell mediated immunity. This association is reported for the first time. The recurrence of solitary keratoacanthoma is a well known but unusual phenomenon which occurs in 5 % of cases (Rook *et al.*, 1967), within 3–6 months of healing of the first lesion. In our case multiple new lesions occurred over a 7-year period.

Usually, keratoacanthomas heal completely. Our patient has had a particularly severe form of keratoacanthoma with mutilation of the left nostril and marked atrophic scars. No squamous or basal cell carcinomas have arisen from the lesions during the course of the disease, as was reported by Burge & Winkelmann (1969) in a case with a solitary tumour treated with methotrexate.

Very few studies of immune defence mechanisms against keratoacanthoma have been made.

Nicolau, Badanoiu & Balus (1963) reported a specific anti-tumour reaction after intradermal injection of an extract of keratoacanthoma. Strong evidence also indicates that cell mediated immunity is the principal effector mechanism in immune defence against tumours (Hellstrom *et al.*, 1971). According to these data, keratoacanthoma should remain isolated and heal spontaneously if the cellular immune responses are efficient. On the other hand keratoacanthomas are known to arise in patients treated with immunosuppressive therapy for kidney transplants (Walder *et al.*, 1971), or in patients with leukaemia (Degos *et al.*, 1967; Weber *et al.*, 1970) or leprosy (Job, 1963; Sebra-Santos & Martins, 1968). Our case shows an alteration of *in vivo* and *in vitro* cellular immune responses without any clinical evidence of infection or of other tumours. Therefore it appears that cell mediated immunity may play a major role in the defence against keratoacanthoma and its deficiency may explain the severity of the evolution of the lesions and their recurrences. Every patient with such keratoacanthomas should be observed carefully and repeatedly for abnormal cellular immune responses, and treatment with immunosuppressive drugs should not be given until new data on the causative agent are available.

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British Journal of Dermatology (1975) **93**, 597.

Comment

CLASSIFICATION AND DIFFERENTIATION OF CUTANEOUS LYMPHOMAS*
Enzyme-cytochemical and immunocytological studies

Traditional classifications of lymphomas are based on clinical or cytomorphological criteria. Progress in enzyme-cytochemical cell differentiation (Burg & Braun-Falco, 1974a) and in our understanding of immunology with respect to the B- and T-lymphocyte differentiation by surface markers and the lymphocyte transformation provide a more functional approach to the classification of cutaneous lymphomas. Studies predominantly of peripheral blood and of lymph nodes have brought some evidence that the proliferating cell in Hodgkin's disease, mycosis fungoides and Sézary's syndrome belongs to the T-cell group (Brouet, Flandrin & Seligmann, 1973; Edelson *et al.*, 1974a; Winkelmann, 1974), whereas chronic lymphatic leukaemia appears to be accepted as a B-cell neoplasm (Grey, Rabellino & Pirofsky, 1971; Preudhomme *et al.*, 1971).

Edelson *et al.* (1974b), by means of immunoglobulin and complement coated erythrocytes on cryostat sections of skin lesions, have shown that most of the cells in mycosis fungoides and in Sézary's syndrome show T-cell characteristics.

It was the purpose of this study to provide a functional approach to lymphoma classification by means of enzyme-cytochemical and immunocytological differentiation of cells in cutaneous lymphoreticular infiltrates and to compare it with the pathogenetic classification in hyperplasia (localized, benign, reactive and reversible proliferation), paraplasia (systemic, malignant and irreversible proliferation) and neoplasia (local tumours of dedifferentiated cells with a tendency to spread by metastases) (Cazal, 1964).

The cellular infiltrates have been evaluated qualitatively and quantitatively in 164 skin biopsies and sixty-one suspensions from homogenized tissue in eighty-eight patients with the following diseases (number of patients investigated in parentheses): Benign lymphocytoma and benign inflammatory reactions from insect bites (24), specific skin infiltrates in chronic lymphatic leukaemia (4), specific skin infiltrates in monocytic leukaemia (1), mycosis fungoides (22), Sézary's syndrome (5), reticulosis (26), reticulosarcoma (6). Enzyme-cytochemical (non-specific esterases, chloroacetate esterase, acid and alkaline phosphatase, leucine aminopeptidase, peroxidase) and immunocytological (immunoglobulin bearing (B-) lymphocytes, spontaneous rosette forming (T-) lymphocytes) methods in conjunction with cytomorphology allow a more reliable identification and differentiation of cells than morphology alone (Burg & Braun-Falco, 1974a).

1. *Benign lymphocytoma* (hyperplasia). The infiltrate is composed of B- and T-lymphocytes in an approximately 2:1 ratio. Large macrophagocytic cells with a high content of non-specific esterases, acid phosphatase and with cytoplasmic inclusions of nuclear fragments ('starry sky cells') are found disseminated within the lymphocytic infiltrate. Some of the lymphocytic cells show alkaline phosphatase activity. In the connective tissue bordering the infiltrate a strong fibroblastic reaction (alkaline phosphatase, leucine aminopeptidase) is obvious (Braun-Falco & Burg, 1975).

2. *Chronic lymphatic leukaemia* (paraplasia). In B-cell leukaemia specific skin infiltrates are primarily composed of B-lymphocytes showing weak surface immunoglobulins but lacking spontaneous rosette formation. There are no 'starry sky cells' within the lymphocytic infiltrate. The fibrous tissue reaction around the infiltrate appears weak.

3. *Monocytic leukaemia* (paraplasia). The specific skin infiltrates consist of a dense accumulation of immature monocytic cells containing NaF-sensitive naphthol-AS-D-acetate esterase, other non-

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* Supported by the Deutsche Forschungsgemeinschaft.

specific esterases and acid phosphatase. Even in clinically uninvolved skin of patients with monocytic leukaemia these cells can be found along blood vessels and adnexal structures.

4. *Mycosis fungoides* (paraplasia). The clinical staging of the disease is reflected in the change of the cellular composition of the infiltrate, showing a more inflammatory character in early stages of mycosis fungoides and a more granulomatous type in the tumour stage. Even in *clinically uninvolved skin* of patients with mycosis fungoides, monocytes are more numerous than in the skin of normal controls. In the *plaque stage* of the disease we find histiocytes, atypical reticulum cells (mycosis fungoides cells), monocytes, granulocytes and up to 50% of enzyme-cytochemically negative round cells with the immunocytological behaviour of T-lymphocytes (spontaneous rosette formation) whereas 10–20% of the lymphocytoid cells bear surface immunoglobulins (B-lymphocytes). In the *tumour stage* of mycosis fungoides the portion of small lymphocytoid round cells vanishes in favour of a proliferation of atypical reticulum cells (Burg & Braun-Falco, 1974b).

5. *Sézary's syndrome* (paraplasia). Besides the main clinical symptoms (erythroderma, pruritus, swelling of the lymph nodes) this syndrome is characterized by atypical cells in the peripheral blood,

TABLE 1. Pathogenetic and functional classification of cutaneous lymphomas

Pathogenetic classification (Cazal, 1964)	Functional classification according to enzymecyto-chemical and immunocytological properties of the cells in skin infiltrates	Number of lesions studied	Average % of lymphocytes:	
			B-	T-
Hyperplasia	I. Lymphocyte predominance			
benign, local reactive, reversible	Mixed B and T lymphocytes: Lymphadenosis benigna cutis (tick bites): B and T lymphocytes; macrophagic 'starry sky cells' (acid phosphatase, nonspecific esterases)	5	42	26
Paraplasia malignant, systemic, irreversible	Predominantly B-lymphocytes: Chronic lymphatic leukaemia: B lymphocytes; 'no starry sky cells'	4	92	4
	Predominantly T lymphocytes: Mycosis fungoides, plaque stage (II): polymorphous infiltrate with predominantly T and with B lymphocytes, atypical reticulum cells, monocytes, granulocytes	6	13	43
	Sézary's syndrome: similar to mycosis fungoides stage II	4	9	35
	II. Mono-histio-(reticulo)cyte predominance; lymphocyte depletion			
	Mycosis fungoides, tumour stage (III): histio-monocytic infiltrate with lymphocytes and granulocytes (especially eosinophils)	7	5	10
	Malignant reticulosis: histiocytes and atypical (reticulum) cells (acid phosphatase and nonspecific esterases), weak or no concomitant inflammatory reaction	9	12	15
	Monocytic leukaemia: monocytes (naphthol-AS-D-acetate esterase, NaF-sensitive)	2	2	1
	III. Undefined (U-, reticulum-?) cell predominance			
Neoplasia malignant starts locally, metastasizes	Reticulosarcoma: Immature cells (acid phosphatase positive, α-naphthylacetate esterase negative, lack of lymphocytic surface markers)	3	16	14

which are probably T-lymphocytes. Functionally identical cells can be isolated from skin infiltrates in patients with Sézary's syndrome just as in the plaque stage of mycosis fungoides.

6. *Malignant reticulosis* (paraplasia). A dense proliferation of nonspecific esterases (except the naphthol-AS-D-acetate esterase) and acid phosphatase positive histiocytic and atypical (reticulum) cells with less than 30% of small lymphocytic round cells is seen. The alkaline phosphatase reaction occasionally may be found positive in some of the small round cells. In a clinically more benign form of the disease—which has a survival time of more than 2 and up to 10 or 15 years, occasionally showing spontaneous remission of some of the lesions—a more pronounced concomitant inflammatory reaction is seen consisting of lymphocytes, monocytes and granulocytes.

7. *Reticulosarcoma* (neoplasia). Here we find a local proliferation of immature cells with a tendency to metastasize. Enzyme-cytochemically the proliferating cells are lacking lymphocytic surface markers and show extraordinarily high acid phosphatase activity whereas alpha-naphthylacetate esterase, which can be found abundantly in monocytes, histiocytes or normal reticulum cells, is completely lacking in these cells.

These studies allow further differentiation of cutaneous lymphomas based on their enzyme cytochemical and immunocytological reaction pattern. Assuming that the predominating cell is identical with the 'tumour cell', with special regard to the involvement of B- and T-lymphocytes, cutaneous lymphoma is classified into a group with lymphocyte predominance, a group with mono-histio-(reticulo)cyte predominance and a third group, where the proliferating cells do not show characteristics of monocytes, histiocytes or lymphocytes and which is referred to as 'undefined'. Table 1 summarizes the most characteristic enzyme-cytochemical and immunocytological features of the lymphomatous skin diseases investigated.

We gratefully acknowledge the technical assistance of Miss E. Ebmeyer and Mrs F. Clauss.

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British Journal of Dermatology (1975) **93**, 601.

Obituaries

THERESA KINDLER

Dr Kindler died on 8 April 1975 a few hours after a fall. She was aged 85.

Her youth was passed mainly in Vienna and she studied in Vienna and Paris. After proceeding Ph.D. in Botany she qualified in medicine in 1923. For several years she worked as a dermatologist in Vienna, later spending some time in Paris at the Pasteur Institute. In 1939, she was helped to come to this country by Dame Louise McIlroy whom she had met in Vienna. On arrival she was registered as an enemy alien and not allowed to practise medicine. However, she lived in the Bermondsey Medical Mission and was able to help in a general way; in due course she had a clinic there and she never lost her interest in its work. After the war (in the blitz, with a fine irony she lost many of the possessions she had been able to bring from Vienna) she took up her dermatological career again. She was SHMO in the Skin Department of the London and Royal Free Hospitals until her retirement and worked also at the Hospital for Sick Children, Great Ormond Street, and at the Queen Elizabeth Hospital for Children, Hackney. For many years subsequently she continued to work on a locum tenens basis. She was known as an able, enthusiastic and highly conscientious dermatologist. She was a member of the British Association of Dermatologists, a member of the Section of Dermatology of the Royal Society of Medicine, and a Fellow of St John's Hospital Dermatological Society, as well as a corresponding member of the Austrian Dermatological Society.

Dr Kindler became a familiar figure in the dermatological life of this country. Frail but indomitable, to the end she rarely missed a meeting and she participated actively in many of them. She contributed several papers to the literature, notably that in 1953 on lichen sclerosus in children. She was of distinguished appearance, her immaculate dress and beautiful complexion commanding admiration. Although a woman of a highly cultivated mind, with wide interests in history and the arts, dermatology—as she frequently insisted—meant everything to her. She could scarcely understand that to any dermatologist it could mean something less, or something else. Her personality was a lively one and she was always kindly, hospitable, and keenly interested in younger dermatologists. Although she often deplored some aspects of present-day life she never ceased to take a pride in the achievements of this country, particularly in the field of dermatology; nor did she forget the part it had played in enabling her to continue her career.

For our part, we salute her integrity and enthusiasm and we appreciate the work she did in her time here. She will be sadly missed, but she was spared a long illness and was able, until her death, to live on as she wished to do in her own familiar and treasured surroundings.

C.M.Ridley

ERNST SKLARZ

Dr E.Sklarz, who died on 6 May, was born at Oels, in Schleswig, on 12 October 1894. He graduated M.D. from the University of Breslau in 1920. From 1920 to 1923 he worked as Assistant to Buschke at the Rudolf Virchow Hospital in Berlin. He then held a number of appointments in Berlin, including that of Director from 1930 to 1936 of the Dermatological Department of the new Polyclinic on the Alexanderplatz. These years were fruitful and his many publications, notably on the influence of the autonomic nervous system on the skin, and pioneer work on Grenz rays in man and in experimental animals made his name widely known.

In 1936 he moved to England for political reasons and the following year obtained the Scottish triple diplomas, LRCP and S (Edin.) and LRFPS (Glasgow). A year later he was appointed postgraduate lecturer in dermatology at the Blackfriars Hospital for Diseases of the Skin, a hospital which played an important role in the development of dermatology in Britain in the nineteenth century. It was there that Jonathan Hutchinson was introduced to dermatology by James Startin and it was there that so many of the leaders of dermatology received their training. Sklarz worked there until the hospital closed, a casualty of the National Health Service. From 1953 until his retirement in 1962 he was consultant dermatologist to the New Cross Hospital, London.

Dr Sklarz accepted with courage and cheerfulness the forcible disruption of an extremely promising career in Berlin, and successfully built up a second career in London, both in his hospital work and in practice and he continued to contribute to the literature of dermatology.

ARTHUR ROOK

British Journal of Dermatology (1975) **93**, 603.

REPORT OF THE MEETING OF THE INTERNATIONAL COMMITTEE OF DERMATOLOGY IN MEXICO CITY, 31 OCTOBER TO 3 NOVEMBER 1974

The International Committee of Dermatology (ICD) of the International League of Dermatological Societies met, in preparation for the XV International Congress of Dermatology which will take place in 1977 in Mexico City. The Committee and its *ex-officio* members Professor Fernando Latapi, Honorary Congress President, Professor Antonio Gonzalez Ochoa, Congress President, and Dr Luciano Dominguez Soto, Secretary General of the Congress, visited the meeting facilities. The Committee also held a special session with the chairmen of the Mexican subcommittees to discuss plans for the Congress. The help of the national dermatological societies and of the investigative dermatological societies is being sought in order to create as interesting and as up-to-date a programme as is possible.

The XV International Congress of Dermatology will take place in Mexico City from 16 to 22 October 1977. The ICD have appointed six sub-committees to help prepare the scientific programmes. With respect to the various activities, the following suggestions were made.

1. *Patient presentations*

Patient presentations by means of colour slides will take place in the large auditorium with simultaneous translations in German, English, French and Spanish. Demonstrations will be limited to 4 minutes and will not be followed by formal discussions. Thus, every day, fifteen patients can be demonstrated allowing for 70 to 75 cases during the Congress. A proportion of these patients will be presented from Latin American countries. The sub-committee plans to ask the national dermatological societies for an early submission of histories and slides so that the best material from all over the world can be selected. There will also be poster exhibits of patients.

2. *Courses*

The XV International Congress of Dermatology will be the first such Congress which will have instructional courses. It is hoped that 50–100 participants will attend each of these courses. The following topics were suggested:

Dermatopathology; Immunology; Photobiology; Structure and function; The dermis and its diseases; Ionizing radiation in dermatology; Physiological treatment of diseases of the skin.

3. *Symposia*

These will be held from 11 to 1.30 daily in large auditoria and will deal with newer developments in dermatology. One to three leaders will be charged with organizing each symposium. Many of the symposia will deal with clinical topics. There will be simultaneous translations. A total of fifteen symposia will have three to five invited speakers, with 30 minutes for each presentation. Time will be left for discussion.

The following are preliminary proposals for symposia:

Syphilis and other venereal diseases; Drug eruptions; Leprosy; IgE anomalies in skin diseases; Connective tissue diseases; Psoriasis; Virus diseases of the skin; Dermatological oncology; Actinic dermatoses; Porphyrias; Vasculitis; Dermatological mycology; Contact dermatitis; Genodermatoses; Acne; Bullous eruptions; Paediatric dermatology.

4. *Guest lectures*

These will take place daily from 10 to 10.45 a.m. in the large auditorium. Outstanding authorities will be invited to talk about problems of general biological interest to dermatologists. The speakers generally will be non-dermatologists. No discussion of these lectures will take place.

5. *Main themes (now called: Topics of current interest)*

The topics of current interest presented by selected dermatologists, will take place in the large auditorium with 3500 seats daily from 11 to 1.30; each presentation will last 30 minutes. The topics will be selected so as to meet the needs of the practising dermatologist.

6. *Workshops*

Workshops are designed for discussions of topics of scientific interest by small groups. Two hundred and fifty different proposals for workshops have already been received by the ICD. From among these, 40 will be selected. These workshops will deal mainly with dermatological basic investigation. Participants will generally be young scientists who wish to discuss special problems of fundamental investigation in a small circle of interested colleagues.

7. *Informal discussion groups*

An attempt will be made to have these in hotels in the late afternoons. If possible, 20–25 such groups will be arranged. The feasibility of holding such discussion groups will be investigated.

8. *Free communications*

A room will be reserved during the Congress for free communications. Some of these will be read only by title.

9. *Audiovisual programmes*

An area in the exhibition hall of the pharmaceutical exhibition will be reserved for audiovisual programmes.

Abstracts of the talks which will be given during the Congress must be furnished by 1 January 1977. They must not exceed 200 words in length and must be in English. The authors of the abstracts are requested to indicate in their abstract whether the paper will be or has been published.

The co-ordinators of the different sub-committees will gather suggestions from the national societies and the dermatological investigative societies, as well as from individuals, and should make these available to the Mexican Organizing Committee.

It was unanimously decided to publish Congress reports of the International Congress in Mexico City. The cost of this report will not be included in the inscription fee for the Congress.

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British Journal of Dermatology (1975) **93,** 605

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ERYTHROPOIETIC PROTOPORPHYRIA: SUBMICROSCOPIC EVENTS DURING THE ACUTE PHOTOSENSITIVITY FLARE

SIR, Gschnait, Wolff & Konrad (1975) in their beautifully illustrated paper describe several puzzling features but fail to take into account one property of endothelium which might explain some of their findings. Damaged endothelium releases activators of fibrinolysis and this is a feature of porphyria as described by Grice *et al.* (1970) and Grice & Ryan (1971). By contrast, in sunburn, fibrinolysis is depressed. Gschnait *et al.* (1975) referred to a paper by Epstein *et al.* (1973) in which the demonstration of 'fibrin' by immunofluorescence was one of the lesser features. 'Fibrinogen' was only occasionally observed. The type of material observed in the study by Gschnait *et al.* (1975) reminds me of degraded fibrin.

Activation of fibrinolysis in porphyria explains why fibrin is not observed in spite of severe small vessel injury. There is no vessel block and very little endothelial proliferation. Failure to form fibrin could explain the poor healing and skin fragility seen in porphyria.

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CORRIGENDA

In the paper by F. Gschnait *et al.* referred to in Dr Ryan's letter, the first author's name was incorrectly printed as Dr F. G. Schnait, for which we apologise. Ed.

ALOPECIA AREATA

SIR, The article of Main *et al.* (1975) on smooth muscle antibodies and alopecia areata in which the author speculates about the autoimmunological origin of the disease gives me the opportunity to draw attention to the work of Tadago Ikeda, *née* Matsubora (1965), on the classification of alopecia areata. In my opinion this is an excellent example of what can be achieved by a purely nosological approach to a syndrome.

In her article, which appeared 10 years ago, she reviewed 1989 patients from Kyoto suffering from alopecia areata. She originally distinguished two forms; in one an individual patch developed slowly, showing cadaverous hairs, black points and reticular patterns of baldness, whereas in the other the patch took less than 6 months to run its course.

The first group was characterized by the relatively frequent occurrence of 'endocrine–autonomic diseases' including diabetes mellitus, peptic ulcer, hypertension, arteriosclerosis, Basedow's disease and myxoedema, and by the presence in many other patients of a history of atopy. These features were absent in the second group. When Ikeda took into account other clinical features of the patients she was able to distinguish four different types of the alopecia syndrome.

(1) The common type, prototype of the 'fast' form, constituting 83% of all cases, occurring between 20 and 40 years, with individual patches lasting less than 6 months and a disappearance of all symptoms within 3 years. The chance of total alopecia in this group was 6%.

(2) The atopic type, prototype of the 'slow' form, constituting 10% of all cases. This occurred in children below 20 years of age, with patches lasting longer than 1 year, the disease persisting for more than 10 years and a 75% chance of total alopecia.

The remaining 7% were divided between:

(3) a 'fast' prehypertensive type, in cases of familial hypertension, and

(4) a 'slow' type combined with endocrine autonomic disorders. The prehypertensive cases occurred at about 20 years of age, with a rather high chance of total alopecia (39%); the fourth type was manifest above the age of 40 and resulted in total alopecia in only about 10% of the cases.

We have been able to confirm Ikeda's figures (Penders, 1967) and we were especially impressed by the high frequency of total alopecia in young children with an atopic background. Müller & Winkelmann (1963), in a survey of 736 patients, stressed the relatively bad prognosis for those with an atopic history. We thus have the impression that the division into the four types has a real significance against the background of aetiology and prognosis. It would be interesting to know how the division of the material in this way would affect the figures in the article of Main *et al.*

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Book Reviews

The Physiology and Pathophysiology of the Skin, vol. 3. The Dermis and the Dendrocytes.

A.JARRETT (Ed.) (1974) London and New York: Academic Press. Pp. 326. Price £13.00.

The third volume in this series deals with the dermis and the dendrocytes. The physiology and pathophysiology of the dermis is written by Dr Jarrett with a little help from Dr Spearman (comparative biology of collagenous tissues) and Dr Ryan (vasculitis). Dr Riley is responsible for six chapters on the dendritic cell population of the epidermis.

Professor Ebling reviewed the first two volumes of the series in this journal last year and many of his comments would also be appropriate for Volume 3. Once again the scope of the work is admirable, though a nightmare for the lone reviewer. I suspect, or rather hope, that there is nobody with a broad enough knowledge to review the whole book objectively.

In the first few chapters Dr Jarrett reaffirms his long held views that the dermis is a gel and not a fibrous network suspended in a ground substance. He considers that the dermis consists of two intimately associated macromolecular systems, collagen and glycosaminoglycans (mucopolysaccharides). The state of the gel depends on the nature of the association between these two components and variations in the polymerization of the tropocollagen molecule. In skin the collagen is relatively unpolymerized and gel-like but the elastic tissue exists as fibres. His hypothesis is supported by evidence pointing out fixation artefacts and ranging from examination of unfixed fresh tissue with intravital fluorochroming to ciné records of the movement of hookworm larvae through the dermis. Some collagen experts may be shocked but Jarrett fans will not be disappointed.

After the chapters on the normal dermis there are sections on the collagenoses and elastoses. A rather small international band of research workers write on lupus erythematosus in dermatological journals. Dr Jarrett's views on the subject are welcomed as they are not along the usual well-trodden pathways. He believes that the basic abnormality in L.E. is most likely an inherited tendency to produce abnormal gammaglobulins. The pathological changes are thought to be due more to their deposition in special sites rather than the result of a specific antigen–antibody reaction against DNA. Scleroderma is considered to be a defect of elastic fibres rather than of collagen, though this seems to be supported by a somewhat partial and incomplete summary of the literature. Nevertheless, Dr Jarrett's views are consistent and, with some ingenuity, he fits them into his general scheme of events in the dermis.

Dr Spearman's chapter on comparative aspects is fascinating. Did you know that nematode worms have a collagenous cuticle produced by the epidermis? You will also be surprised to learn that your non-synthetic bathroom sponge is in fact a highly polymerised form of collagen.

Dr Riley gives a most comprehensive and authoritative account of the pigmentary system. Comparative aspects of pigmentation and the embryonic origin of melanocytes are discussed particularly well. The biochemistry of pigment formation is not only understandable but related logically to disorders of melanin metabolism. The chapter on the Langerhans cell is more standard though tables summarizing evidence for and against various theories on the cell's nature are helpful. It is concluded that the cell probably influences keratinization and that Langerhans cells and melanocytes, if related at all, may compete for similar epidermal territories. If this chapter was written today perhaps more emphasis would have been placed on an immunological function of the cell. The chapter on melanoma, except for the section on the aetiology of the tumour, is disappointing.

Many reviews on the previous volumes in this series have praised the reference system. It is so convenient that one wonders why references cannot be included at the bottom of each page of all such books. There is, however, a real rash of printing errors. They include almost everything that a final check should have eliminated. Misprints galore, spelling mistakes, words left out, references to the wrong figure and description of figures in black and white as though they were in colour. One trusts that there was more attention to detail in the research described in the text.

So far these comments have not indicated the real value of this book. Once again Dr Jarrett and his contributors have shown that there is more to dermatology than just labelling and treating diseases. Readers will not fail to be impressed by their continued attempts to explain what is happening in diseases and to make appropriate comparisons in animals. It may be too late for many dermatologists to change their ways but trainees, in particular, should read this book because of its enlightened approach.

J.A.A.HUNTER

Clinical Aspects of Immunology. P.G.H.GELL, R.R.A.COOMBS and P.J.LACHMANN (Eds.) (1975) 3rd edn. Oxford: Blackwell Scientific Publications. Pp. 1754. Price £21.50.

Clinical immunology has come to stay and dermatology along with other branches of medicine and surgery has benefited tremendously from the understanding of disease mechanisms it has provided. The first edition of this standard 'classic' appeared in 1963. Since then the number of its pages has grown from 816 to 1725, the number of contributors has increased to seventy-two, nearly half of them new since the second edition, and the two highly distinguished editors have been joined by Peter Lachmann. They could hardly have chosen more wisely.

In their foreword to this edition the editors emphasize that this volume is in part supplementary to, rather than substituting for, the second edition, which should be used in parallel with this one—certainly a novel and economical approach to a universal problem.

All contributors have made notable contributions to their chosen field of interest and have been able to review clearly and concisely the principal methods, issues and implications of their subject. The expert will find the information and references he seeks and even the most humble clinician, whose training in immunology had been confined to patch testing and the tuberculin reaction, will find much of this book compulsive reading and an invaluable source of reference. Of particular interest to dermatologists are the chapter on allergic contact dermatitis, in which Calnan's large clinical experience is now ably supported by Turk's immunological expertise, Pepys' masterly canvas of the whole spectrum of atopy, Lachmann's guide to the intricacies of auto-allergy and the most stimulating exposition by Champion and Parish of allergic diseases in the skin. Highly relevant to dermatology are also topics such as the immunological aspects of diagnostic histopathology, immunity in malignant disease, the immunoglobulins, immunity deficiency states, the clinical pharmacology of immunosuppressive drugs, immunity in malignant disease, and the immunological mechanisms in drug hypersensitivity. Of rapidly increasing importance is the subject of HL-A antigens and their significance, clearly and comprehensively presented by Joysey, who has also managed to append 118 references up to (and including) 1973.

The editors and all contributors deserve great credit for their work and our gratitude for having produced the best book in this popular field. It is as essential to dermatologists as is 'Rook' (*Rook's Textbook of Dermatology*).

G.Holti

Nail Diseases in Internal Medicine. Pietro de Nicola, Mario Morsiani and Giorgio Zavagli (1974) 1st edn. Springfield, Illinois: Charles C. Thomas. Pp. 113, illustrated. Price $10.50.

The purpose of this monograph is to present a concise survey of the most pertinent aspects of nail disorders in internal medicine, in contrast to previous texts which have paid particular attention to nail abnormalities in skin diseases. In fact only approximately one-third relates to nail changes in internal disease, the rest being anatomy and physiology and nail symptoms, as in previous books relating to this subject. Most of the colour illustrations appear at the beginning of the book and are of poor quality; this, plus the very bad English and unwieldy classification of symptoms make for difficult reading.

In its present form therefore, the English edition of this book cannot be recommended, either to physician or dermatologist.

R.Dawber

Annual Review of Allergy 1973. Ed. by Claude Albee Frazier (1974) New York: Medical Examination Publishing Company. Pp. 535. Price $15.00.

This king-sized paperback is ring-bound and has rather closely printed pages. It is divided into thirty-two chapters comprising either review articles or a series of abstracts of recently published work. The emphasis is on clinical aspects of immunology but basic work is included. The chest is well represented and there are sections on tumour and transplantation immunology, food allergy, allergy to arthropods and a useful chapter on drug allergy.

A twenty-one page essay on Immuno-dermatology is written by Philip Blank. The topics covered are atopic dermatitis, contact dermatitis and urticaria, and there are 126 references. Dr Blank is to be congratulated on bringing together in readable form a synopsis of a large mass of hetereogeneous information.

It is most useful to have a regular volume of this kind in which recent work in an expanding field is annotated. This is the second edition and I hope there will be many more.

G.M.Levene

The Systemic Manifestations of Inflammatory Bowel Disease. Ed. by William B. Luckash and Raymond B. Johnson (1975) Springfield, Illinois, U.S.A.: Charles C. Thomas. Pp. 356.

This book derives from the proceedings of a Symposium concerning the 'Complications of Inflammatory Bowel Disease' held at the annual meeting of the Association of Military Surgeons of the United States. Thirty authors have contributed chapters on the diagnosis, treatment, intestinal and extra-intestinal complications of the inflammatory diseases of the bowel.

W. Taylor Johnson and William N. Narva from the Department of Dermatology, National Naval Medical Center, Bethesda, devote nine pages to the main skin complications of ulcerative colitis and regional enteritis. These include pyoderma gangrenosum, aphthous stomatitis, pyostomatitis vegetans, fistulae, abscesses, ulcerating erythematous plaques and papulonecrotic lesions. There are three colour plates of pyoderma gangrenosum and a black and white illustration of a papulonecrotic lesion.

The information contained is probably fairly well known to most dermatologists but should be helpful to the general physician.

H.T.H.Wilson

Proceedings of the XIVth Congress of the Association of French-speaking Dermatologists and Syphilologists, Geneva, 1973 (1974). Geneva. Medicine et Hygiene. Vol. I., Toxicodermies, pp 403. Vol. II, Vascularites, pp. 233.

It was ambitious indeed to attempt to cover two such extensive subjects at a single time. The results well justify the effort, even though the presentations and short communications inevitably show the unevenness associated with large meetings and numerous speakers—forty-one relating to the drug eruption alone. In both fields, events are moving fast and new techniques and methods of measurement are enlarging our knowledge of the mechanisms involved while creating new problems of their own. Reviews written in 1973 may well be outdated by 1975. Nevertheless, when comprehensive and backed by adequate references, they remain extremely useful as 'key articles' to which we turn with profit. We cannot all be research workers and I suspect that those who are will find such reviews as useful stepping stones in a turbulent stream. The contents of the two volumes fall into three groups—review articles, original experimental work and clinical observations. The main papers are arranged in a logical order but the short communications, presumably published in the order of presentation, might perhaps have been arranged more advantageously.

In Volume I, the drug eruptions are dealt with in considerable detail. A well-documented classification is followed by an authoritative account by Thivolet and his colleagues on the immunological mechanisms, with useful tables of the frequency of the clinical and immunological presentations. Reactions to penicillins are presented in detail by Revuz and Touraine, the histological features by Duperrat and Mme Noury-Duperrat. Puissant and his collaborators present an interesting comparison of the immunological tests available for identification of drug eruptions and the problem posed in young children is examined by Desmons—a useful contribution in a difficult field. Several papers were devoted to unusual causes of toxic epidermal necrolysis and its complications, including a 'dry, subcorneal' variety (by Stevanovic), which will further complicate the identification of the subcorneal bullous eruptions.

In Volume II the leukocytoclastic form of vasculitis dominates the scene, though cryoglobulinaemia in relation to vasculitis is discussed by Guilaine, and Behçet's syndrome in two other papers. The EM changes in allergic vasculitis are presented clearly and succinctly by Olmos and his colleagues in a well-illustrated paper. The section on treatment deals with all the forms of therapy known in this country—and a few more.

The clinical presentations include some little known forms, e.g. a particular form of juvenile gangrenous vasculitis of the scrotum by Pinol Aguade and his colleagues, a vesicular and bullous form of erythema elevatum diutinum and two papers on Sweet's syndrome.

Though English readers with even a slight grasp of French will have little difficulty in understanding the text and the many excellent Tables, it was encouraging to find Piñol Aguadé, at least, adding a short summary in English. Such a practice would, I am sure, increase the attraction of important reviews of this type for workers in other countries. I warmly recommend these two volumes both to clinicians and to investigative workers. Though much of the material may be well known to them, these volumes incorporate a considerable body of experience and the references, which are often very comprehensive, are themselves a valuable guide to the work that has been done in these difficult areas.

D.S.WILKINSON

British Journal of Dermatology (1975) **93**, 611.

News and Notices

A Symposium on Methods for the Measurement of The Physical Properties of the Skin

A Symposium on Methods for the Measurement of the Physical Properties of the Skin will be held under the auspices of The University of Wales (Ronnie Marks) and University of Miami (Harvey Blank) 10–14 March in Miami, Florida. Attendance will be limited, but those who would like to contribute or participate are invited to correspond with either Dr R. Marks (The Welsh National School of Medicine, Department of Medicine (Dermatology), Heath Park, Cardiff CF4 4XN) or Professor Harvey Blank (Department of Dermatology, University of Miami, Miami, Florida U.S.A. 33136).

E.S.D.R. Symposium—Dermatitis Herpetiformis

Department of Dermatology, University of Lund, Friday 16 January 1976. This is the first of a series of clinically orientated symposia to be held by the E.S.D.R. The symposium will consist of a series of short papers from invited speakers, illustrative case presentations, discussion periods and a session for free communications. Anyone wishing to contribute to or attend this meeting should contact either Professor H. Rorsmann, University of Lund, Sweden, or Dr R. Marks, University Hospital of Wales, Cardiff.

Annual Meeting of E.S.D.R. 1976

The 1976 Annual Meeting of the European Society for Dermatological Research will take place in the RAI Congrescentrum in Amsterdam on 26–28 April, 1976. Attendance at the scientific sessions will be open to all members, contributors and guests. Non-members who wish to present a paper are kindly requested to submit an abstract. They will be invited to attend the meeting as either contributors or guests. Abstract forms and information about the meeting can be obtained from:
Ch. M. Lapiere, E.S.D.R. Secretary, Department of Dermatology, Hôpital de Bavière, B-4000 Liège, Belgium (tel. 041-43.93.72).

INTERNATIONAL SYMPOSIUM ON PSORIASIS

A Second International Symposium on Psoriasis is to be held on 12–16 July, 1976 at the Department of Dermatology, Stanford University, Stanford, California, 94305, U.S.A. For information, please write to: Eugene M. Farber, M.D., Director, Paul H. Jacobs, M.D., Co-Director or Alvin J. Cox, M.D., Co-Director.

APPOINTMENTS

South Glamorgan

Dr Arthur Grice Knight has been appointed Consultant Dermatologist to the South Glamorgan Health Authority. Dr Knight graduated M.B., Ch.B. (Sheffield) 1964 and became M.R.C.P. (Edinburgh) with neurology as a special subject and M.D. with commendation 1973. His postgraduate training took place in Sheffield in the Departments of Dermatology and Neurology and later he was a Research Fellow in Dermatology in the Scripps Clinic and Research Foundation, California from 1971 to 1973. His most recent appointment has been Senior Registrar and Tutor at St John's Hospital, London and he has experience in diagnostic immunofluorescence techniques. His publications include several papers on mycology and he continues to take an interest in mycological research.

Bradford Area

Dr Tahir Saeed Haroon has been appointed Consultant Dermatologist to the Bradford Royal Infirmary and Associated Hospitals. Dr Haroon qualified B.Sc. from Punjab University in 1958, M.B., B.S. from Punjab University in 1961 and became M.R.C.P. (UK) in 1973. His training in General Medicine was at the Royal Albert Edward Infirmary, Wigan, Lancashire and in Dermatology he was trained at the Stobhill Hospital, Glasgow, and later at the Glasgow Royal Infirmary.

Gloucestershire Area

Dr Thomas Joseph Delaney has been appointed Consultant Dermatologist to the Gloucestershire Health Area. He qualified M.B., Ch.B. from Liverpool in 1964 and obtained his MRCP (London) 1968 and DCH (London) 1968. His training in General Medicine and Paediatrics took place in Liverpool Hospitals and he started training in Dermatology in the Liverpool Infirmary. He eventually became Senior Registrar and Tutor in Dermatology in the United Liverpool Hospitals. In 1971 he moved to the Skin Department of St George's Hospital, London and did a Clinical Assistantship at St John's Hospital for Diseases of the Skin. He has written a large number of papers of clinical interest and has also taken part in the preparation of many audiovisual dermatological teaching aids.

Clinical and Experimental Dermatology

This Journal covering all aspects of Dermatology will succeed the Transactions of the St John's Hospital Dermatological Society from March 1976, and will appear quarterly. It will be published by the same publishers as the British Journal of Dermatology. The two Journals will remain independent of each other with some liasion between the editors.

Dermatologica

Offizielles Organ der Schweizerischen Gesellschaft für Dermatologie und Venerologie
Organe officiel de la Société Suisse de Dermatologie et de Vénérologie
Official Organ of the Nederlandse Vereniging van Dermatologen

Editors: *J. Delacrétaz* (Lausanne); *W. Jadassohn* (Geneva); *L.H. Jansen* (Utrecht); *M.K. Polano* (Leiden), and *R. Schuppli* (Basel)

Index Vol. 150, No. 4, 1975

Proceedings · Verhandlungsberichte · Congrès

56. Jahresversammlung der Schweizerischen Gesellschaft für Dermatologie und Venereologie, 18. und 19. Oktober 1974 in Lausanne – 56e Réunion annuelle de la Société Suisse de Dermatologie et de Vénéréologie, les 18 et 19 octobre 1974 à Lausanne

Mitteilungen · Communications

Brun, R. (Genève): Statistique des tests épicutanés positifs de 1000 cas d'eczéma de contact (Epidemiology of Contact Dermatitis in Geneva [1,000 Cases])

Bader, C.; Orusco, M. et Laugier, P. (Genève): Recherche systématique de la blennorragie dans une consultation de planning familial
(Value of Screening for Gonorrhea at a Family Planning Consultation)

Abstracts

Demonstrationen · Démonstrations

Rufli, Th. (Basel)

Laugier, P.; Hunziker, N.; Orusco, M.; Brun, R.; Reiffers, J. et Posternak, F. (Genève)

Delacrétaz, J.; Frenk, E. et Tapernoux, B. (Lausanne)

Kaufmann, J.; Gilliet, F.; Ott, F. und Wüthrich, B. (Zürich)

Krebs, A. und Mitarbeiter (Bern)

Pillai, K.G.; Singh, G., and Sharma, B.M. (Varanasi): *Trichophyton rubrum* Infection of the Penis

Subscription date: 'Dermatologica' appears monthly in numbers of approximately 64 pages each. 6 numbers form 1 volume and 2 volumes are issued annually. Subscription price per volume SFr. 96.– / US $ 35.00 / DM 91.– / £ 14.40

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Journal of the Society of

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of Great Britain

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VOL. 26 NO. 7 JULY 1975

E. W. CLARK. Estimation of the general incidence of specific lanolin allergy.

E. J. CLAR, C. P. HER AND C. G. STURELLE. Skin impedance and moisturization.

C. CROPPER, P. PLATT AND N. A. PUTTNAM. Analytical procedures for the determination of chlorhexidine in oral products.

The *Journal of the Society of Cosmetic Chemists* is published twelve times a year at £30.00 per annum, post free. New subscriptions and requests for specimen copies of the journal should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents.*

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations,* A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of the Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: Cunliffe, W.J. & Shuster, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: Beare, J.M. & Wilson Jones, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 93, Number 5, November 1975

CONTENTS

Clinical and Laboratory Investigations

PAGE

Natural Antibiosis Among Skin Bacteria as a Primary Defence Against Infection 487
SYDNEY SELWYN
Ultrastructural Localization and Differentiation of Membrane-bound ATP Utilizing Enzymes Including Adenyl Cyclase in Normal and Psoriatic Epidermis 495
G. MAHRLE AND C. E. ORFANOS
Lysosomal Hydrolases of the Epidermis. 3. Peptide Hydrolases 509
P. D. MIERAND JOSÉ J. M. A. VAN DEN HURK
Livedo Reticularis. Signs in the Skin of Disturbance of Blood Viscosity and of Blood Flow 519
P. W. M. COPEMAN
The Significance of a Positive Cutaneous Immunofluorescence Test in Systemic Lupus Erythematosus 531
PAUL I. DANTZIG, JACQUELINE MAURO, SYRUS RAYHANZADEH AND ULRICH H. RUDOFSKY
Cellular Immunity and Circulating Antibody to Herpes Simplex Virus in Subjects with Recurrent Herpes Simplex Lesions and Controls as Measured by the Mixed Leukocyte Migration Inhibition Test and Complement Fixation 539
R. W. GANGE, A. DE BATS, J. R. PARK, C. M. P. BRADSTREET AND E. L. RHODES
In Vitro Assay of Immunity to Human Wart Antigen 545
WARWICK L. MORISON
Cell-mediated Immune Responses in Patients with Warts 553
WARWICK L. MORISON
Nickel Hypersensitivity. Nickel Binding to Amino Acids and Lymphocytes 557
F. HUTCHINSON, T. M. MACLEOD AND E. J. RAFFLE

Pharmacology and Treatment

Comparative Bio-availability and Activity of Proprietary Topical Corticosteroid Preparations: Vasoconstrictor Assays on Thirty-one Ointments 563
B. W. BARRY AND R. WOODFORD
Treatment of Pemphigus with Cyclophosphamide 573
J. S. PASRICHA, V. D. SOOD AND Y. MINOCHA
A Follow-up of Tetracycline-treated Rosacea with Special Reference to Rosacea Keratitis 577
ARTHUR G. KNIGHT AND C. F. H. VICKERS

Case Reports

Hodgkin's Disease in a Patient with Follicular and Dermal Mucinosis and Spontaneous Ooze of Mucin (Mucinorrhoea) 581
L. STANKLER AND S. W. B. EWEN
Leprechaunism. Case Report in a Black African Child 587
R. G. HARTDEGEN, M. DOGLIOTTI, L. RABINOWITZ AND R. G. BARTLETT
Multiple Keratoacanthomas: Association with Deficient Cell Mediated Immunity 593
A. CLAUDY AND J. THIVOLET

Comment

Classification and Differentiation of Cutaneous Lymphomas. Enzyme-cytochemical and Immunocytological Studies 597
GÜNTER BURG AND OTTO BRAUN-FALCO
Obituaries 601
THERESA KINDLER AND ERNST SKLARZ

Report

Report of the Meeting of the International Committee of Dermatology in Mexico City, 31 October to 3 November 1974 603

Correspondence

Erythropoietic Protoporphyria: Submicroscopic Events During the Acute Photosensitivity Flare 605
T. J. RYAN
Alopecia Areata 605
J. W. H. MALI
Book Reviews 606
News and Notices 611

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British Journal of Dermatology

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References

1. Brit. J. Derm. **92**, 585, 1975
2. Archs. Derm. **86**, 608, 1962
3. Brit. J. Derm. **90**, 197, 1974

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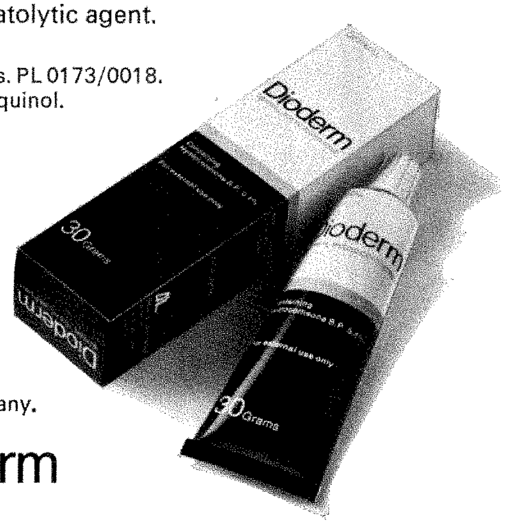

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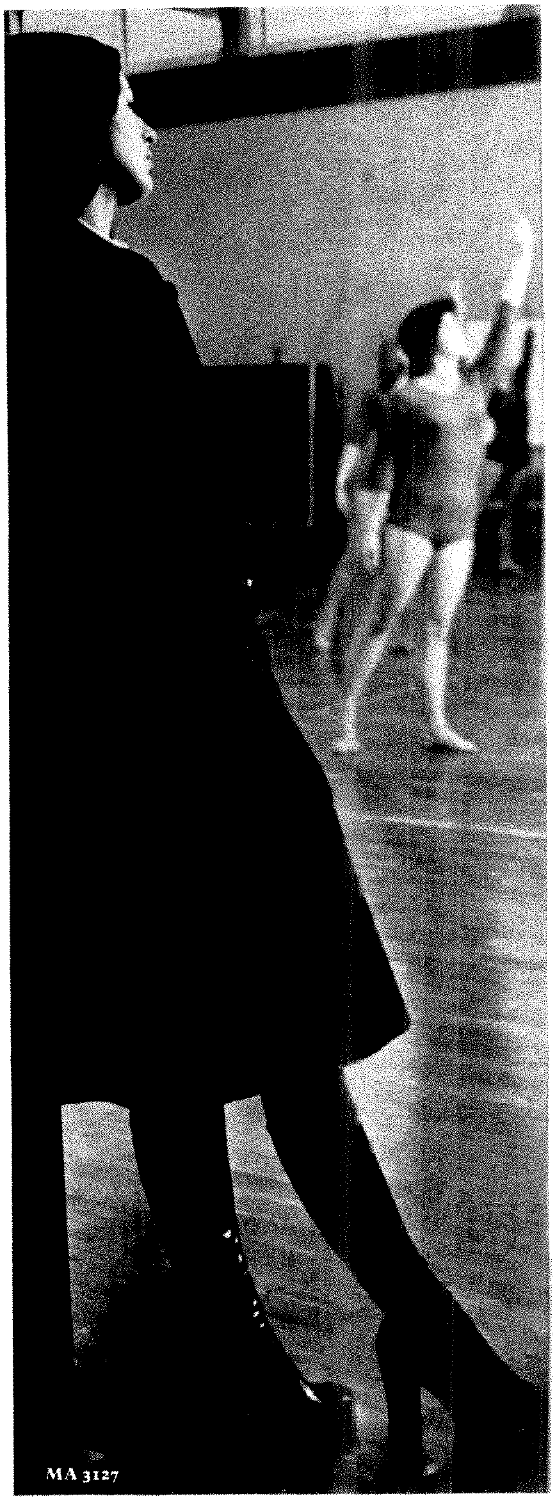
*British Medical Journal (1975) **3**, 626



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1. Nathanson, L., et al., Clinical Pharmacol. Therap. **12** (6) 955-962 (1971)
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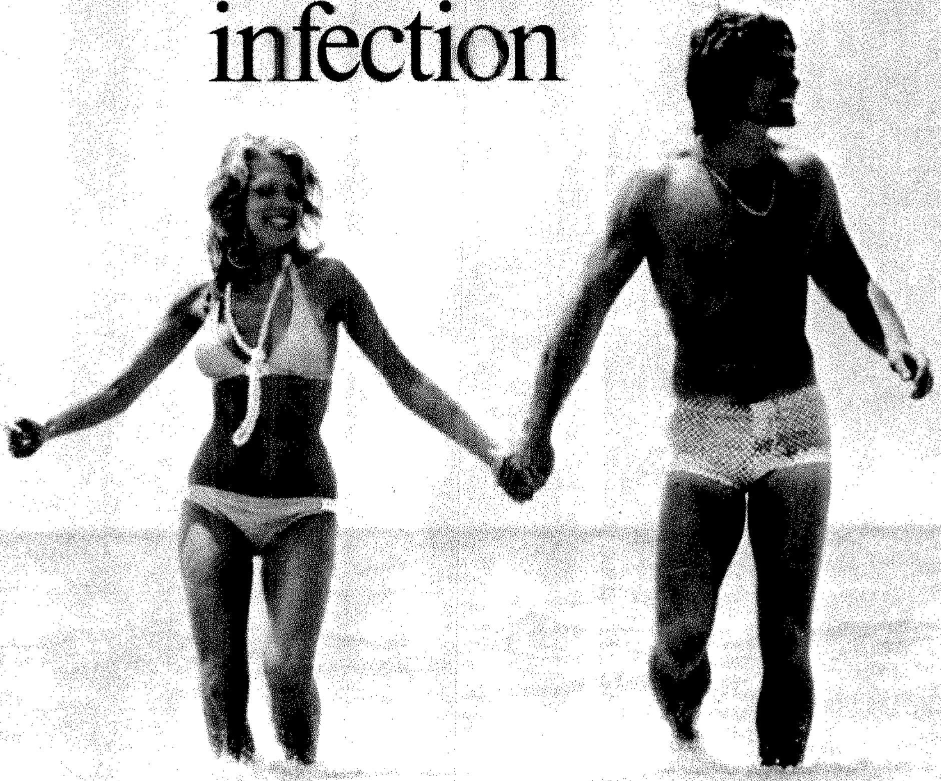

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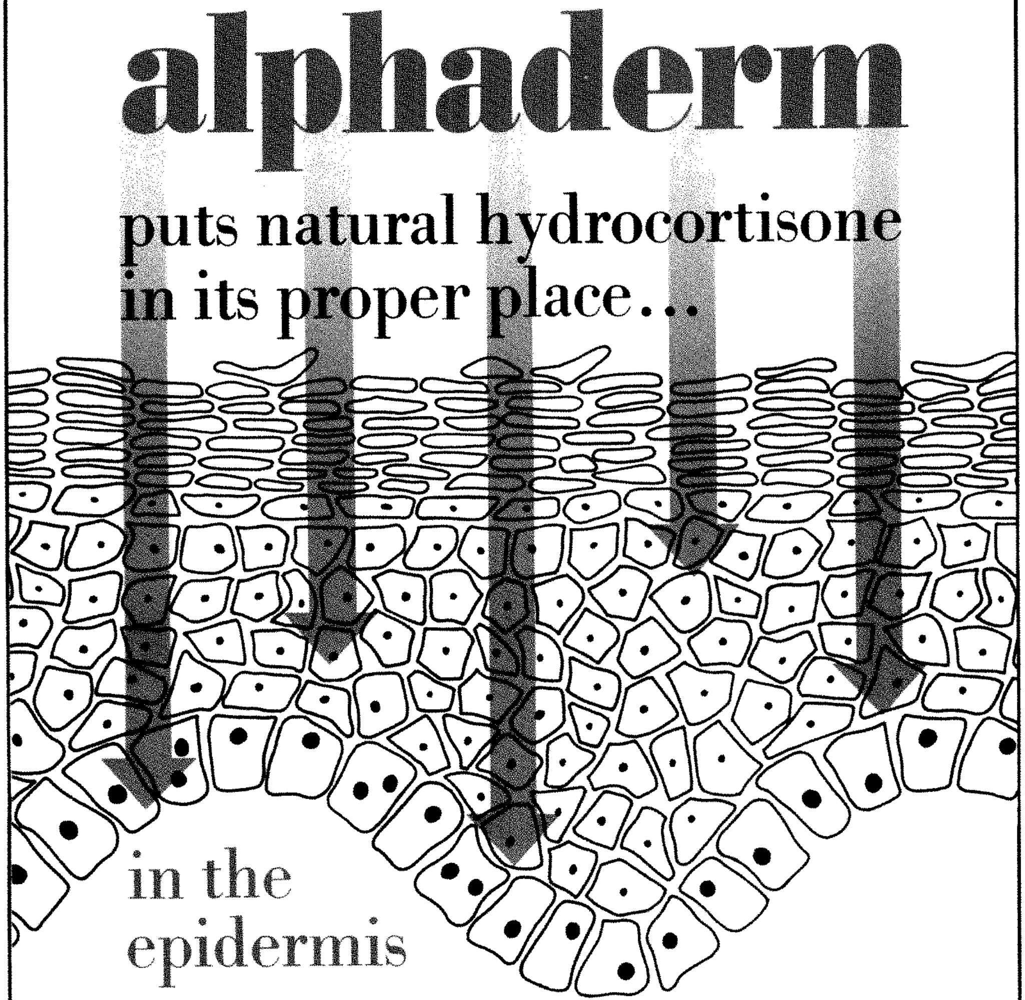

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References
1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91**, 579
2. Jacoby, R.H. and Gilkes, J.J.H. (1974) *Curr.med.Res.Opin.* **2**, 474

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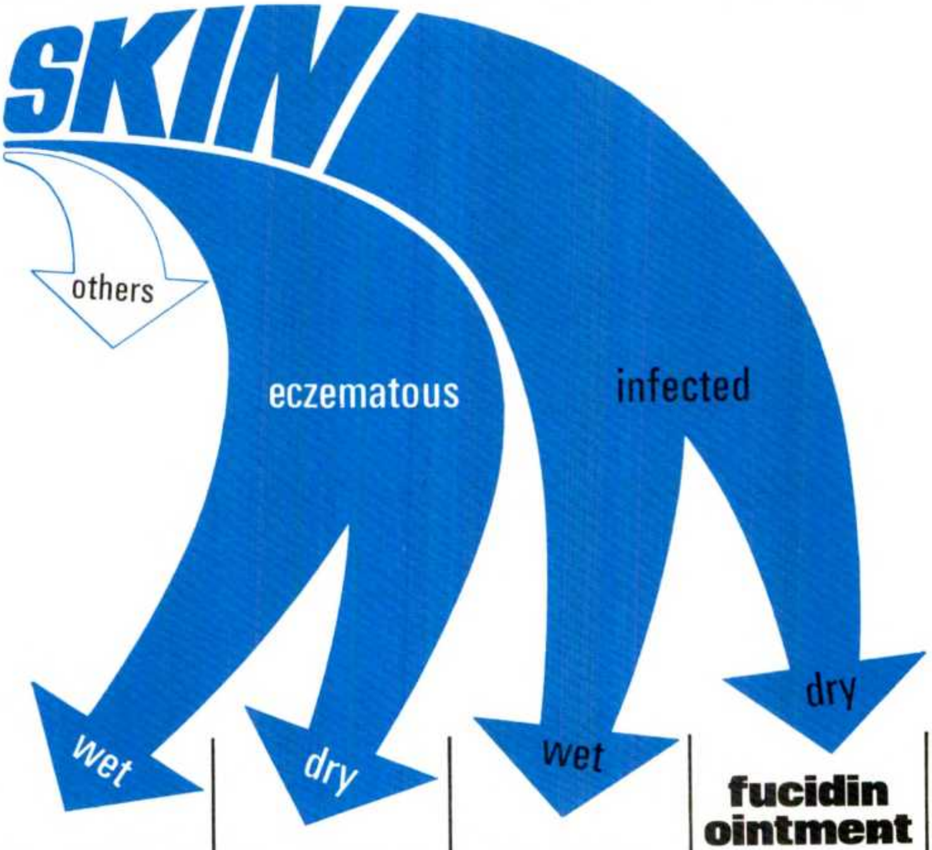

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Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

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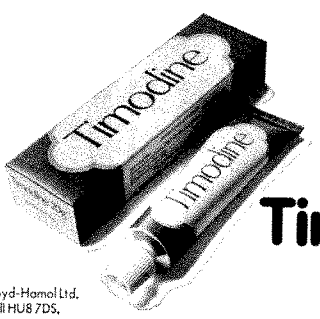

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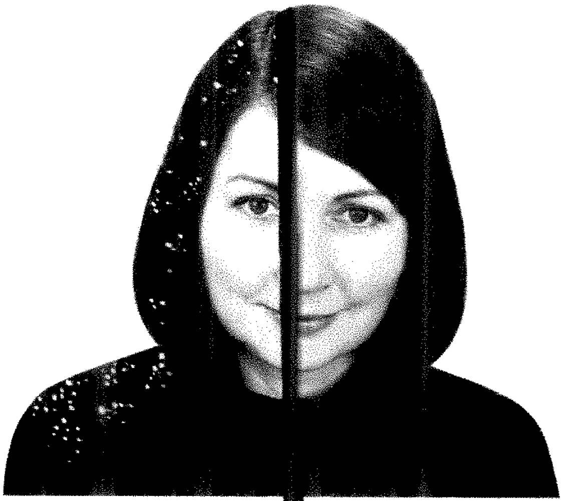

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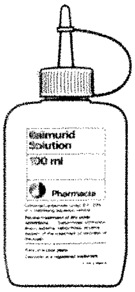

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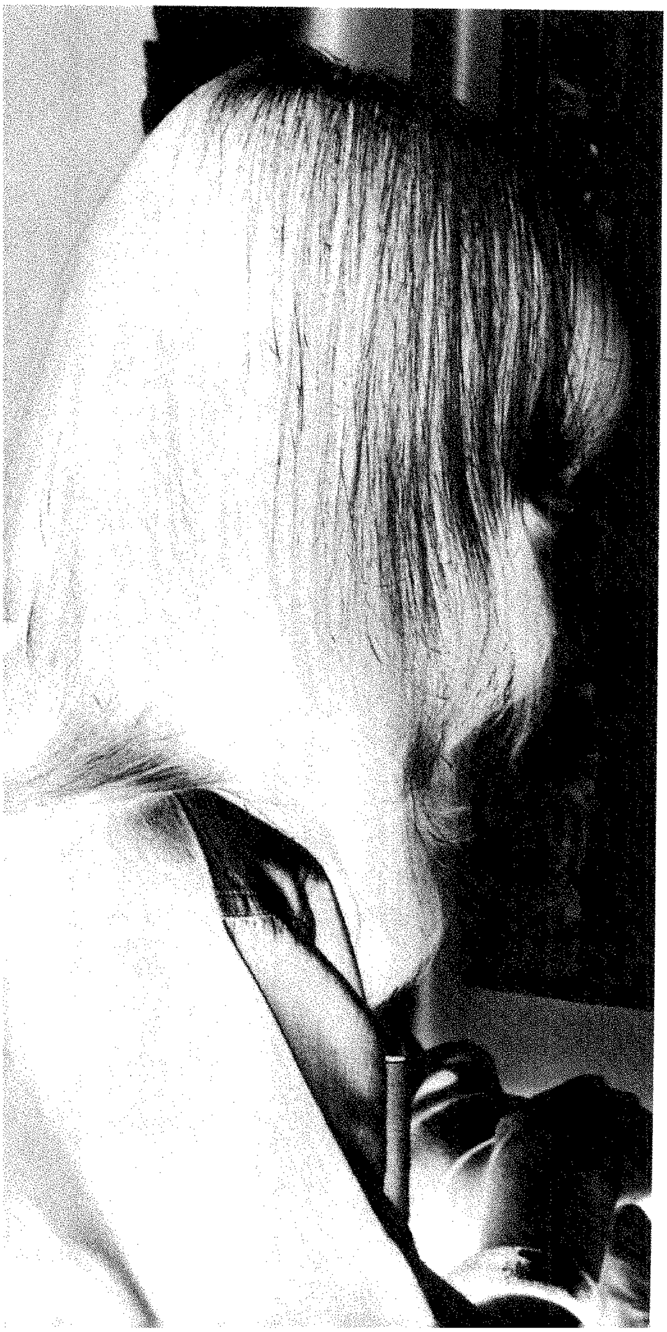

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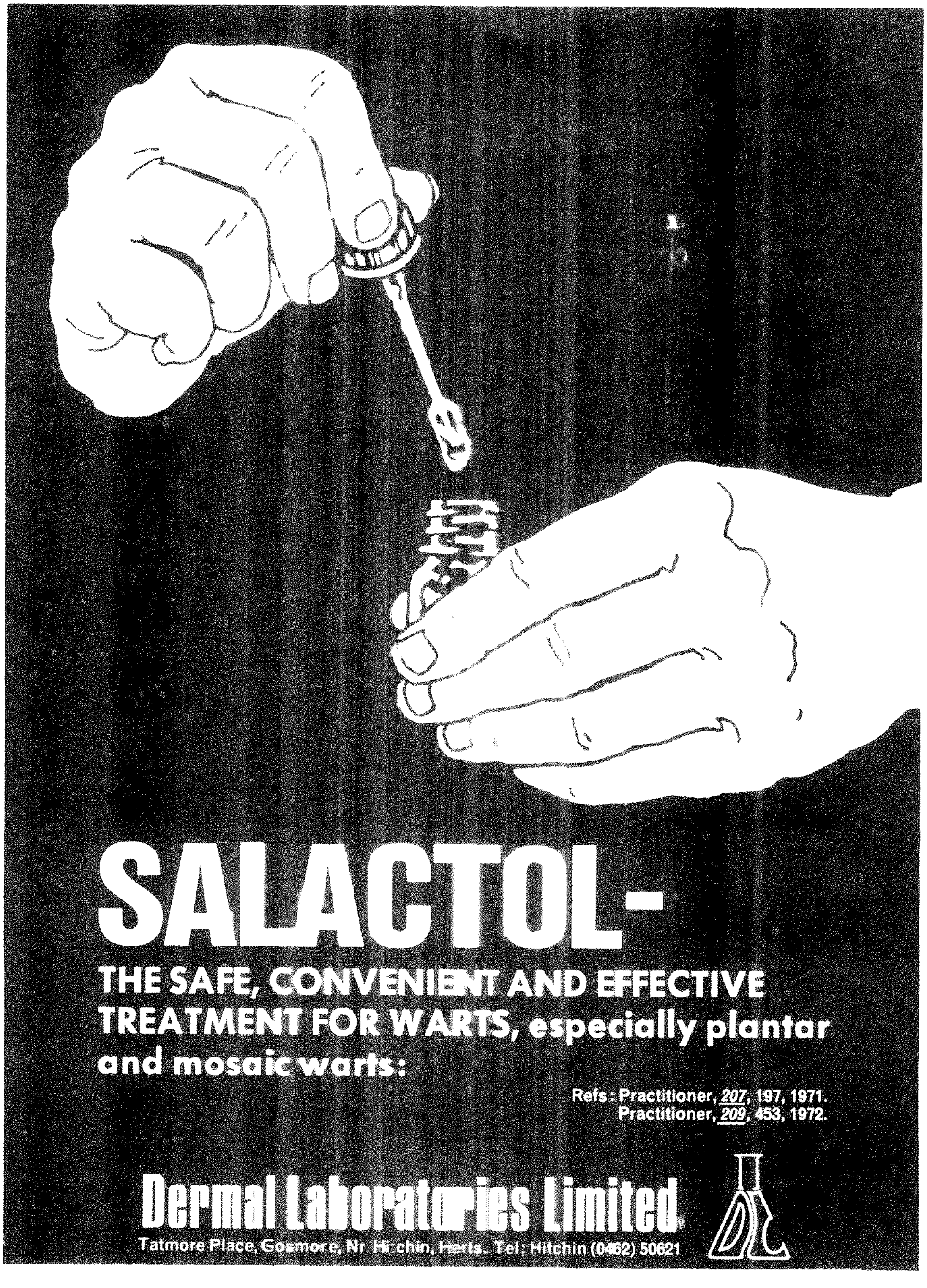

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British Journal of Dermatology (1975) **93,** 613.

Exogenous ochronosis and pigmented colloid milium from hydroquinone bleaching creams

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*Department of Dermatology, and †Department of Pathology, University of Pretoria, South Africa

Accepted for publication 17 March 1975

SUMMARY

An outbreak of ochronosis and colloid milium is described after the use of strong hydroquinone bleaching creams. These phenomena developed only after a few years, and took place when the melanocytes had overcome the bleaching influence. Sun-exposure and thorough inunction of the cream were required for the more advanced changes. Analogous changes have been seen when the skin is exposed to certain crude fuels in individuals working in the sun, and phenolic components in the fuels are suspected. The study presented here covers the clinical, histological, histochemical, electron microscopical and pathogenetic features as seen in thirty-five cases of hydroquinone damage to the dermis in South Africa.

Hydroquinone-containing bleaching creams are effective and much sought after by South African Negro women. Their success is easily demonstrated by comparing the bleached face with the untreated natural dark brown of the neck or dorsal surface of the hand (Plate 1a). The cytological changes of melanocytes under the influence of hydroquinone have been studied by Jimbow *et al.* (1974), and the pharmacology of acute hydroquinone bleaching is now evidently well grounded.

We wish to describe a process in which the facial epidermis overcomes this bleaching effect during treatment, and may grow darker than normal particularly over certain sun-irradiated areas. Over those parts where the cream can be well rubbed in, the darkening tends to be most intense. Thereafter, novel changes develop in the papillary dermis. This layer becomes ochronotic together with colloid degeneration and colloid milium production. These alterations may be observed while parts of the face retain their bleached appearance, though as a rule the initial bleaching effect of the cream is lost. Some manufacturers have included a sunscreen in the cream, but for those patients who are much in the sun, its incorporation has not forestalled the deeper changes.

Our awareness of this disorder was slow to develop. Over a 5-year-period (1969–1974), a series of some thirty-five cases was selected and studied. The influx of new cases still continues, and we could have doubled the number of patients for investigation. The striking development of pigmented colloid milium, in which the face looks spotted with caviar, was first recognized by Alan Menter during 1969 to 1971. The dermal ochronotic features were then studied by G.H.Findlay. The rare ochronotic changes in the ear cartilage were discovered by J.G.L.Morrison, who also first noted ac-

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quired colloid milium from sun-exposure when the skin was soaked in power paraffin. Our understanding was delayed in the early 1970's by an epidemic of monobenzone leukomelanoderma affecting perhaps 1000 cases. Only after chemical analyses (Findlay during 1974) of certain creams with approximately 5% hydroquinone and more, were we able to distinguish them from those containing monobenzone, and relate our novel findings to the effects of hydroquinone. A campaign against monobenzone has perhaps succeeded. Now we are left principally with the hydroquinone reactions, though we still see both, sometimes even combined in the same individual.

The use of bleaching face creams by black women is not confined to that part of the Third World where our study was done. Their appeal depends on a strange allurement of tradition, aesthetics, advertising, money and power. There is a current saying, for example, in the Pedi language: 'Go away in your blackness', which has still to be replaced by the slogan from Francophone Africa, 'Black is beautiful'. The artful advertising of these skin lighteners would need a study on its own, but it would be mistaken to see the choice as lying between black and white. It is an alternative of light brown as against a dark brown skin.

Clinical features

The thirty-five patients studied were adult black (South African Bantu) women who had used strong hydroquinone-containing creams continuously for several (up to 8) years, and developed the dermatosis gradually over ½–3 years as a rule, or longer. Because of the slow emergence of the disorder, the maximum age incidence among the patients was rather high—between 30 and 39 years.

Colloid milium (Plate 1b and c) was the grossest and most persistent change. The visible units consisted of clustered or confluent tense papules and micropapules, mostly darker than normal skin, being a patchy grey colour down to a glistening jet black. Between them the skin creases were preserved and otherwise normal. Where the milia lay farther apart, the intervening skin could suggest a worm-eaten atrophy, or look normal amid the network of papules. Scars were free of milia. The lesions were distributed in sharp zones corresponding to areas with firm underlying tissues, the commonest site being the outer bony margin of the orbit from the eyebrow to the zygoma. The second commonest was the cheek area over the masseter and least common the posterior triangle of the neck. The centre of the face was sometimes mildly affected. The evolution of the lesions required sun-exposure, being limited by head covers and wigs and accentuated over the more skywardly directed parts of the zygoma. The lesions were also distributed where night creams could remain trapped, e.g. in eyebrows, creases round the eyes, cavity of the pinna. No other part of the body was ever affected. Individual lesions could involute after years as judged by serial photographs, but the bulk were unchanged.

Pigmentation with textural change (Colloid degeneration, ochronosis, elastosis) (Plate 1d). Where milia were not prominent, the same areas of distribution could show a reticulated and ripple-like, sooty pigmentation. Along areas of milium formation, the ripply spots became expanded into papular

PLATE 1. (a) Successful bleaching of the face with hydroquinone cream compared with the untreated natural dark brown skin of the dorsal surface of the hand.

(b & c) Clustered glistening pigmented colloid milia with clear patches among the papules. Overall and close-up views.

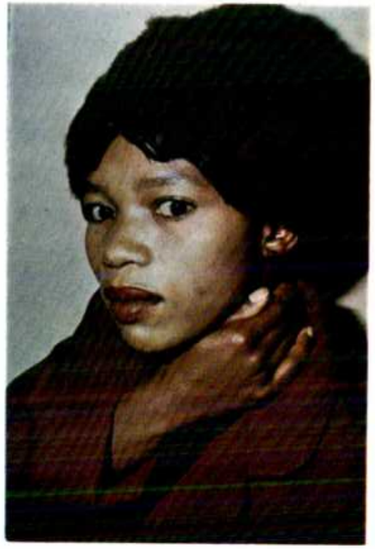
(d) Reticulated and ripple-like darkening with early papular milia over the bony margin of the orbit. The pigment represents a combination of melanosis and ochronosis.

(e) Early focus of ochronotic and elastotic fibres in the papillary layer (Magnification × 100). Haematoxylin and eosin.

(f) Ochronotic fibres among a mass of broad dense collagen bundles (Magnification × 100). Haematoxylin and eosin.

facing p. 614

(a)



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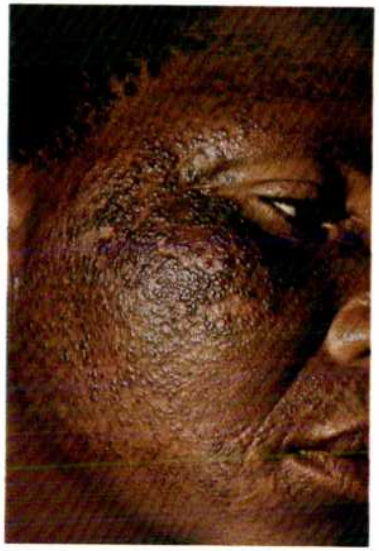

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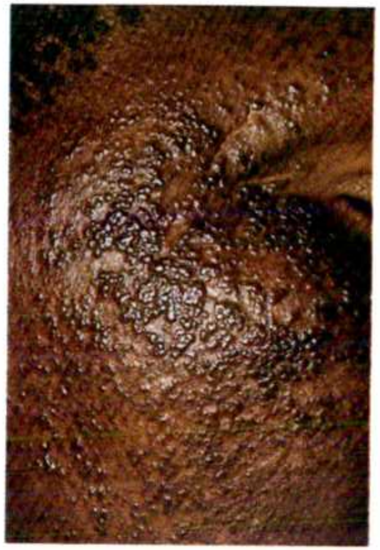

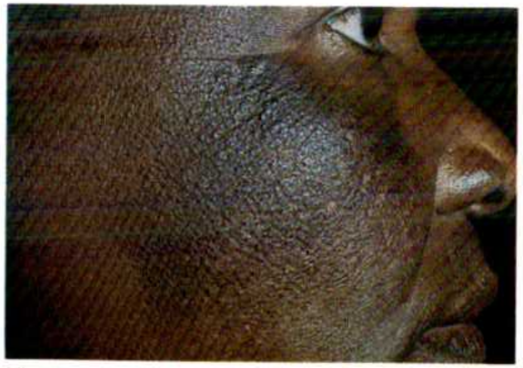

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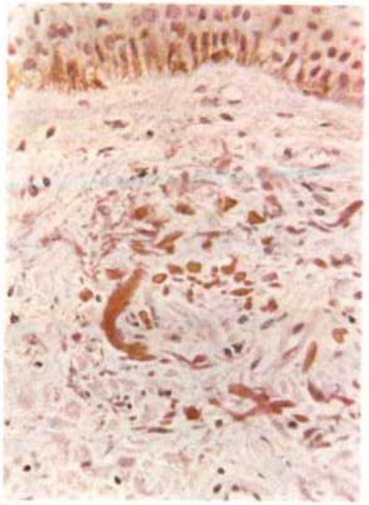

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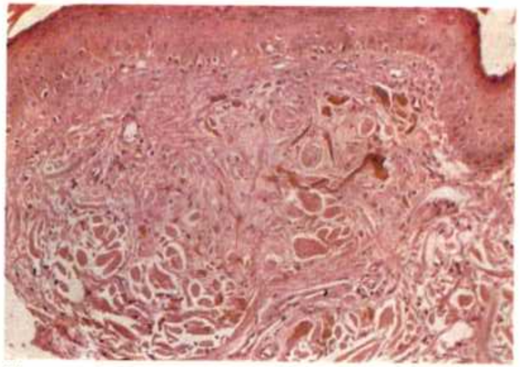

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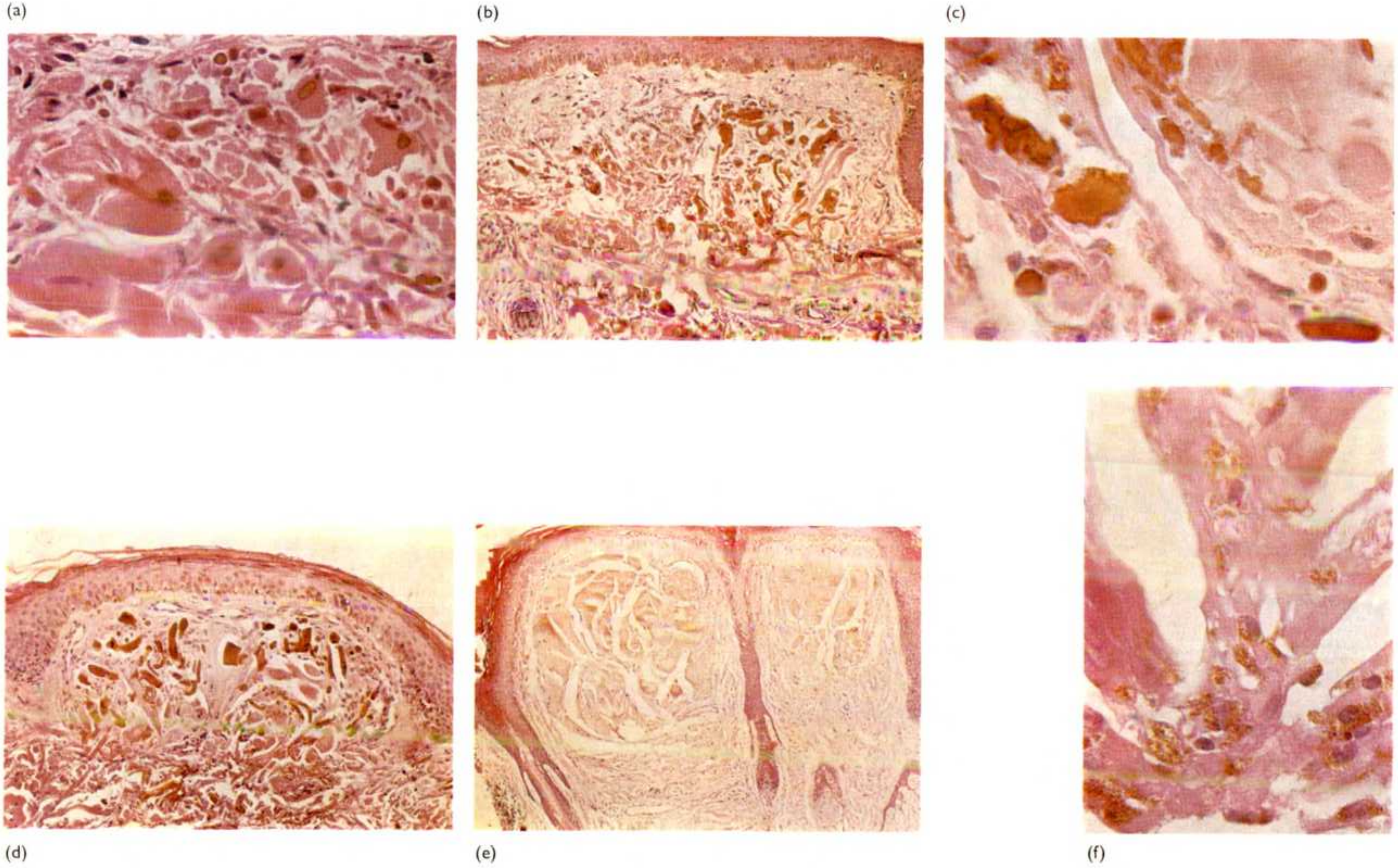
(a)
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milia. By stretching the ripply folds, paler normal skin was seen in between. Sometimes the pigment was diffuse on the sun exposed areas. There was great histological variety among these changes even when the appearances were clinically uniform.

Ear cartilage ochronosis. A blue tint in six cases with colloid milium was observed in the cavity of the auricle. It could have been overlooked in our earlier cases.

Further clinical comments. Hair growth and colour were unchanged. Other pigmentary changes were observed, such as chloasma, freckly marks and post-inflammatory pigmentation. These were not part of the bleaching cream dermatosis so much as the reason for its being first used.

In those patients re-examined after a year or more the dermatosis was not much altered. Some subsidence may have followed the prohibition of skin lightening creams, the use of a sunscreen by day and a steroid cream at night, but in general the changes were not strikingly reversed. Some patients had even tried the same bleach again for the persisting darkness of the skin. In one case followed for 5 years the milia were successfully removed with a skin graft knife, leaving a mildly hyperpigmented smooth skin.

Pathology

Forty-eight skin biopsies from thirty-five patients were studied by standard histological methods, and ten blocks from three patients by electron microscopy. Five biopsies were made of the skin and cartilage of the auricle. The main lesions occurred in the papillary layer of the dermis, which showed focal expansions containing ochronosis, elastosis, abnormal collagen with colloid degeneration, colloid milium, pigmentary changes, telangiectasia with stretched plexus vessels around the tissue expansions, and sundry interstitial changes. Between the abnormal foci and below them there was little amiss, and epidermal changes were unspectacular.

Ochronosis was common to all specimens (Plate 1e and f). The most striking ochronotic elements were broad stumpy fibres, parallel sided, curled or banana shaped, obviously tough, apt to tear and jump out of the sections, tending to transverse fracture, with jagged or pointed extremities. The fibres were yellow-brown, but this natural shade was obscured by many connective tissue stains. A mild eosin stain interfered least with the ochronotic colour. Near the surface the fibres were bigger and darker, and inclined to have sharp black edges. In the middle and deeper papillary layers the fibres were narrower, more yellow or olive-green, and sinuous, like elastotic fibres, merging below at the subpapillary plexus with ordinary elastotic fibres. Some small, more greenish ochronotic bundles were surrounded by an eosinophilic cuff looking like fibrillar collagen, elastic or some nondescript cracked hyaline material (Plate 2a). Structures resembling altered collagen bundles sometimes showed a dif-

---

PLATE 2. (a) Yellow-green ochronotic bundles surrounded by an eosinophilic cuff of hyaline material (Magnification × 500).

(b) Group of partially degraded ochronotic fibres (Magnification × 100).

(c) Ochronotic bundles undergoing degradation. The fibre surfaces are cavitated and surrounded by ochronotic granular material and amorphous residua. Dendritic, pigment-laden cells are abundant (Magnification × 500).

(d) Early milium formed by hyaline masses, ochronotic fibres, amorphous residua and pigment-laden cells (Magnification × 100).

(e) Fully developed colloid milia with lightly pigmented ochronotic colloid blocks (Magnification × 100).

(f) Cracked colloid masses containing amoeboid cells packed with granular pigment (Magnification × 500).

fuse brown colour, and least frequently the same tint was observed in the colloid in the colloid milium elements. There was no ochronosis in most of the elastic and collagen fibres or in the interstitial spaces.

Ochronotic fibres became degraded (Plate 2b and c). The larger ones could appear cavitated on the surfaces with a granular eosinophil material in the indentations, thus converting a regular-shaped fibre into a miniature jigsaw puzzle piece. Medium sized fibres developed irregular lumpy ochronotic deposits, frayed out or moth-eaten appearances, or blow-outs into amorphous masses. Only rarely was there ingestion of these chunks by foreign body giant cells or foam-filled phagocytes.

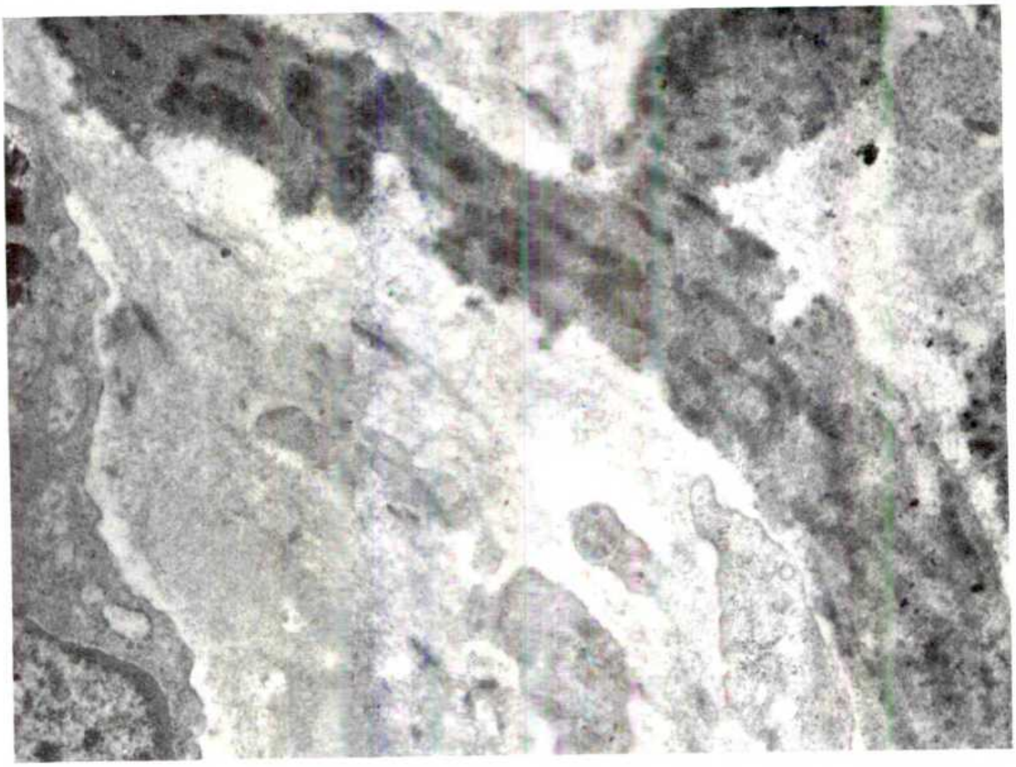

FIGURE 1. An ochronotic fibre containing electron dense areas in a pale amorphous matrix with a moth-eaten outline. (Electron micrograph × 22,500.)

The well-formed ochronotic fibres contained electron-dense areas in a paler amorphous matrix. Their outlines were irregular and moth-eaten (Fig. 1). When the fibres disintegrated, the dense granular masses were left surrounded by amorphous colloid material. Collagen bundles were quite distinct from them.

Ochronotic fibres were PAS-negative, often positive with elastic stains, negative with argyrophil methods which stained epidermal melanin only, but argentaffin-positive with the Fontana and Schmorl's ferricyanide methods. Epidermis and associated melanocytes over ochronotic areas were usually normal though heavily pigmented. Pigment incontinence was regularly present. In two instances, ochronotic fibres were in the course of trans-epidermal extrusion.

In the ear cartilage, ochronosis with fibrillar degeneration and chondrocytes filled with pigment granules were obvious in the outer third of the cartilage. The effect was selective, since the mid- and inner thirds of the cartilage were more normal, and overlying dermal ochronosis virtually absent (Fig. 2).

Solar elastosis was often seen associated with ochronosis, was rare in colloid milium except right underneath the milium suggesting an earlier process, and was absent in lesions dominated by swollen ochronotic collagen bundles.

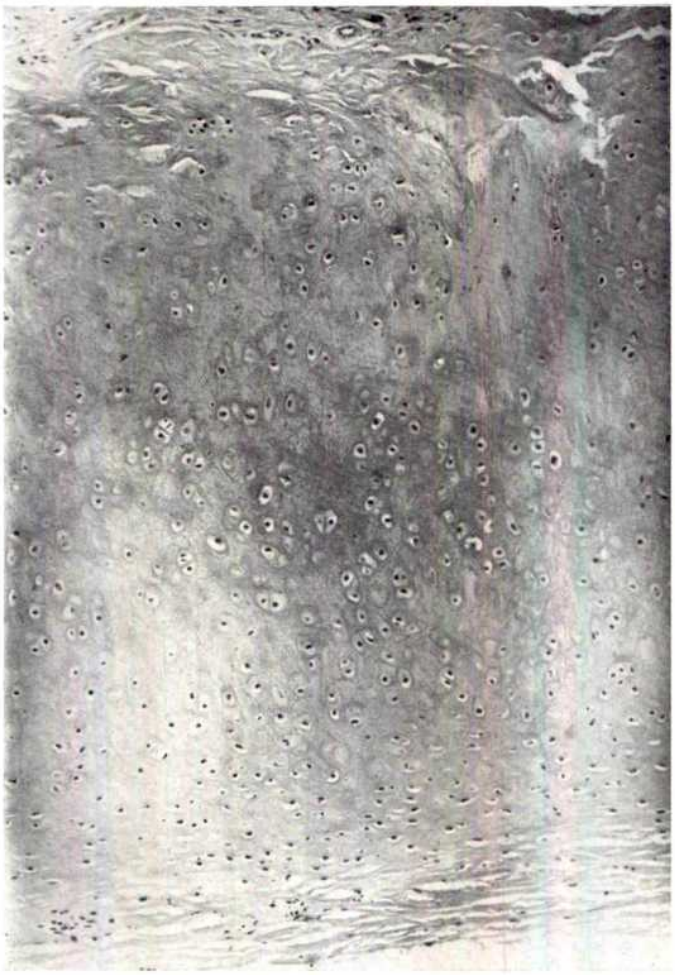

FIGURE 2. Section through the entire ear cartilage, with the lateral face above showing ochronotic disintegration while the medial face below has normal cartilage and perichondrium.

Collagen bundle abnormalities and degeneration. Papillary collagen bundles could differ mainly in being broad and straplike. These thick masses sometimes looked keloidal, and could become acellular and burnt-out while remaining in bundles (Plate 1f). Other bundles were swollen, shredded and spongy, losing the bundle pattern and fusing into pale colloid. Sometimes this clot was eosinophilic, with cells in between like osteoid tissue. Small tangles or whorls of collagen were seen very occasionally. Abnormal bundles sometimes appeared to push up fanwise to the surface from the base of the papillary zone and to be more prone to decay nearer the epidermis. Diffuse ochronosis in them was variable.

Colloid milia (Plate 2d and e). The colloid part of the fully-developed colloid milium in our patients looked precisely like colloid milium as otherwise understood. In many sections the eosinophilic, cracked colloid masses contained no visible ochronotic material. Our full range yielded a variety of intermediate phases in the construction and destruction of colloid milia. Colloid change was naturally more marked towards the outside of the papillary layer and in the longer-established cases. An intermediate sort of colloid change showed ochronotic semi-colloid blocks with great swelling of degenerate collagen bundles as they enlarged and fused to produce this change. Wherever ochronosis was still obvious there were very few cells present. The lower parts of the milium tended to be spongy as in the degenerating collagen bundles. There was also the 'osteoid' type of change intermediate between collagen degeneration and colloid milium. Some expansions occurred and fusion of elastotic and swollen ochronotic fibres with the main colloid mass. This contribution was minor. The ochronotic component of a colloid milium, when identifiable, was mostly in the diffuse and seldom in the rod-shaped fibre form.

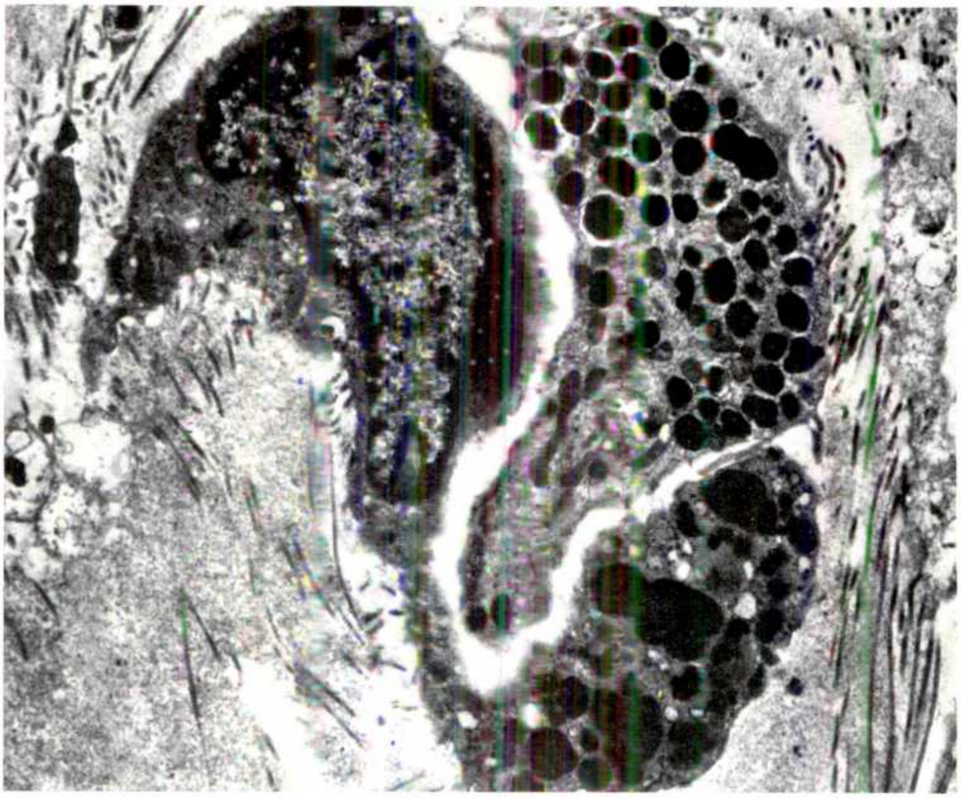

FIGURE 3. Dendritic cells lying in the amorphous matrix of the colloid milium containing scattered collagen fibres. (Electron micrograph × 11,880.)

Colloid milia were transected and surrounded by capillaries, and were highly cellular in the peripheral and intervening spaces round the colloid material.

Electron microscopically the colloid consisted of an amorphous matrix containing scattered cross-striated collagen microfibrils (Fig. 3).

The milium material failed to stain with the Fontana or Schmorl methods and failed to fluoresce with thioflavine-T.

Over the milium the epidermis was usually thin and stretched. In only three cases did the basal layer contain less or no pigment, and four showed liquefaction degeneration.

Major cell components. Although some areas of ochronotic collagen and colloid were cell-poor, the cell population in the papillary dermis was diffusely increased. Despite some concentration round the subpapillary plexus, none of the cells was perivascular or grouped. Most cells lying between ochronotic bundles or colloid were of either dendritic or amoeboid shape (Plate 2f). In the milia they lay in phagocytic lacunae like osteoclasts. On smears of the milia the nuclear structure was, according to C.S.Kingsley (Hematologist), like that of bone marrow phagocytes. Giant cells with few nuclei were found near dense colloid or ochronotic material.

In all these interstitial cell types, pigment granules, larger than epidermal melanosomes, and staining positively for melanin with silver procedures and Schmorl's method, were commonly seen in the cytoplasm. Others showed a granular or foamy cytoplasm.

The source of the pigment and nature of the cells were problematic. Judging from their position the melanin-type pigment could most readily arrive in the cells from the decaying ochronotic deposits nearby, from which it would be laid down in granular form inside the cells. As an alternative source the epidermal melanocytes were rather remote, and the pigment incontinence in their vicinity was not comparable with the cell changes considered here. A polymerized hydroquinone derivative of more directly external origin is another possibility, though none of these alternatives excludes the others.

The ultrastructural appearance of the dendritic ramifications of the apparently amoeboid cells was most impressive. Numerous cytolysosomes contained electron-dense material, presumably the melanin (Figs 3 and 4). Others contained a substance similar to the colloid outside the cells. Fine ribosomes, granular endoplasmic reticulum and cytoplasmic filaments were present, and many cells had dilated vesicles of granular endoplasmic reticulum in the cytoplasm in which abundant lysosomes were also present (Fig. 4). Occasionally an appearance strongly suggesting phagocytosis was seen. The frankly dendritic cells also contained phagocytosed 'melanin' in the cytolysosomes, cytoplasmic filaments and a ballooned granular endoplasmic reticulum. These appearances suggested that the cells had both fibroblast and macrophage functions, and were responsible for collagen formation as well as a partial clearance of ochronotic fibres and colloid milia with pigment phagocytosis.

Minor features. Mast cells were present but exhibited no special relation to the milia or other abnormal components. Inflammatory changes were not in evidence. Despite the oedematous swelling of the lesions there was no necrosis or notable cellular reaction. Fibroblasts in the basement membrane zone were unchanged, though this zone was sometimes thickened. In the tissue spaces, unidentified foamy, fibrillar and granular material of considerable variety made its appearance, evidently in relation to fibre bundle disintegration.

Pathogenesis

The ensuing remarks summarize our conclusions from a survey of the microscopic material.

Melanocyte recovery. Melanocytes have probably overcome the damaging influence of hydroquinone, and are overactive. Prolonged application of the drug with sun stimulation appear to lead to this result. Since changes in the dermis are not seen during the normal bleaching action of hydroquinone, their appearance after melanocyte recovery suggests that the selective and damaging uptake of hydro-

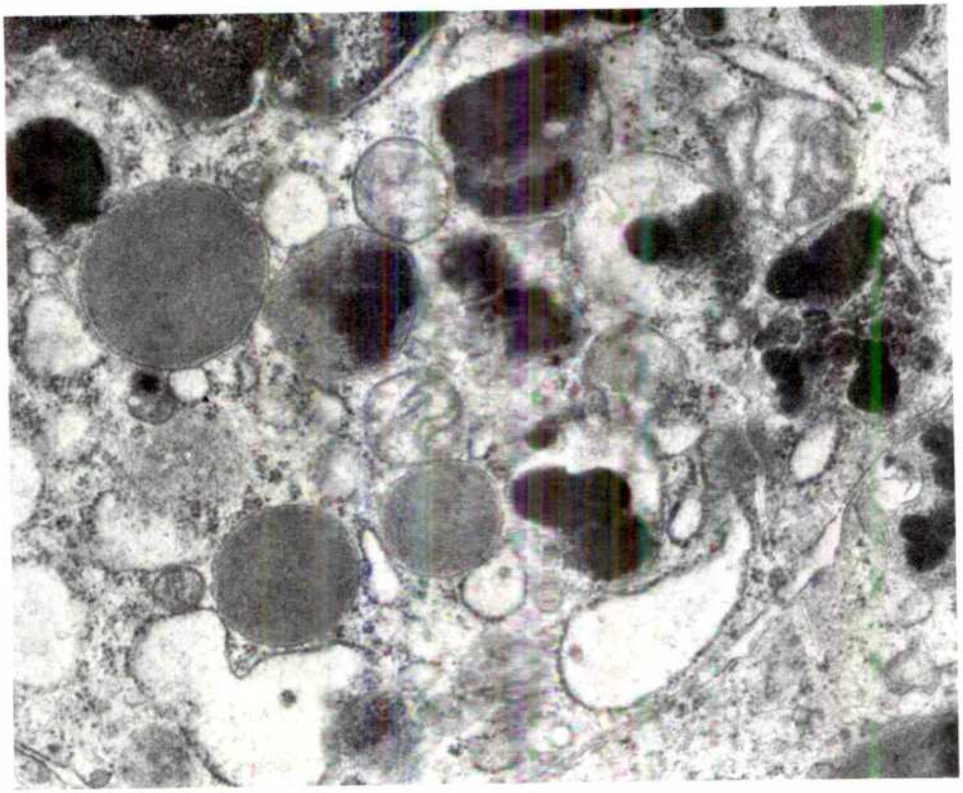

FIGURE 4. Cytolysosomes containing electron dense material, ribosomes, dilated vesicles of granular endoplasmic reticulum and microtubules in the cytoplasm of a dendritic cell. (Electron micrograph × 36,000.)

quinone by melanocytes has ceased, and the drug with any of its chemical and metabolic derivatives are now passed down to the papillary dermis.

Fibroblast function. The dermal papillary fibroblasts possess two differing functions. The superficial set, largely unaffected here, maintains the basement membrane zone and adjacent subepidermal layer. The deep set, partly concentrated in groups near the subpapillary plexus, are most probably employed in fibrovascular tissue maintenance and repair. In hydroquinone poisoning under the influence of sunlight these cell groups appear to produce the radiating complexes of abnormal fibres resembling collagen which deteriorate further as they approach the surface. Ochronosis in these fibres is diffuse and mild.

In the intervening areas between the subepidermal and the lower sub-papillary sets of fibroblasts, many scattered fibroblasts fail to produce such conspicuously focal lesions. These are the cells among which phagocytosis may evolve.

Elastotic factor. The elastotic fibres in the lower or mid-papillary dermis in a dark individual would require some factor derived from sunburnt epidermis to induce their production. Quite probably these fibroblasts which form the elastotic fibres are exposed to more than one such epidermal influence. Hydroquinone or its derivatives could alter the process by disturbing the redox requirements of fibre production.

Hydroquinone factor. The structure of elastin permits the attachment and polymerisation of many phenols in the interior of the fibre (Partridge, 1970) and this mordant-like action could occur with hydroquinone. A theory in which hydroquinone acts as a simple intra-vital fibre mordant of elastin fibres would not explain the other fibre changes, nor their focal nature. We would favour an active uptake of the phenolic ochronosis precursors by fibroblasts which will not only alter elastic fibre production but allow an excretion of the abnormal material into the central regions of newly forming fibre bundles.

Pigment phagocytosis. The suggestion is made that ochronotic collagen, when being transformed into the colloid of colloid milium, could yield its pigment to a phagocytic cell in the vicinity.

COMMENTS

A minor epidemic of ochronosis from bleaching creams is hard to trace to its sources, but in 1966 certain commercial preparations containing 3% hydroquinone were strengthened to 6 and 8% and put in a more adherent and penetrating base. It was from 1969 onward that we became increasingly familiar with this new disease.

Ochronosis, whether brought on by phenol, resorcinol, quinone vapour, 2,5-hydroquinone acetic acid (homogentisic acid), experimental force-feeding of tyrosine, etc. requires an initial glut of the substance in some parts of the organism. This overload affects connective tissues in ways which lead to deposits of pigmented polymers, at the same time bringing about an accelerated ageing of the tissues.

Our study brings certain phenomena into prominence. Ageing of facial skin from sun exposure, and certain sun-induced changes which may be genetically determined, i.e. colloid milium, are here brought on synergistically by hydroquinone. Where sun exposure is not needed for damage to occur, as in the case of the ear cartilage, hydroquinone brought about the destructive change unaided, while the overlying skin escaped.

Our large yield of biopsy material supported the view that the abnormal fibres and other components were newly produced and thereafter more susceptible to extracellular alteration. Such later alterations led to two results. The altered material occupied its place in the tissue semi-permanently, largely escaping from the normal connective tissue turnover. This same material would evidently act as an exchange centre for diverse organic and inorganic materials, such as ochronosis pigment, serum components, water, etc. thus accounting for a great diversity in its reported composition. The cells encompassing the colloid milium masses and fragments were able to exchange components with the milium and other abnormal fibrous material, but were seldom able to destroy them or to form them in full.

Our findings with hydroquinone differed in principle but little from those seen with genetically determined alkaptonuria. Hewitt, Wegmann & Guignon (1962) and Bazex & Dupré (1962) favoured an active or dynamic nature of alkaptonuric lesions in connective tissue in their limited case material—a view which our work fully supports. We have therefore avoided the misleading expression 'degeneration', as suggesting an unfavourable change in pre-existing and previously normal extracellular connective tissue components.

We have tried to resolve the nature of hydroquinone-induced colloid milium by proposing that it is principally a collagen-derived exchange polymer of an irremovable kind, surrounded and interpenetrated by a loose cordon of dendritic and phagocytic cells. This view is broader than one proposed in recent studies on true colloid milium (Hashimoto, Miller & Bereston, 1972) in which the very similar looking surrounding cells are regarded as fibroblasts somehow producing the whole milium.

It differs even more from an earlier view of our own (Findlay, 1954) that the milium was derived entirely from altered elastotic fibres.

The closest parallel to what we describe was reported by Holzberger (1960) from the Netherlands Antilles. There a crude fuel (gas-oil), used to clean the hands during hard manual work in the sun, caused an epidemic of colloid milium confined to the hands among labourers in an oil refinery. We asked Dr J.P.de Villiers of the National Chemical Research Laboratory, Pretoria, if such gas-oil would contain phenols. He replied, quoting Littman and Lochte (*The Petroleum Acids and Bases. New York: Chemical Publishing Co.* 1955) 'Apparently phenols are present in all petroleum fractions of the proper boiling point range' (p. 120). Needless to say (he continued) gas-oil is a fraction with the proper boiling point range. More interestingly they mention on p. 134 that trimethyl hydroquinone has been isolated from Texas oil, but that little work has in fact been done on phenols in petroleum oil as the difficulties of isolation make this an economically unacceptable source. In my opinion (he concluded) you are justified in postulating that the Caribbean and South African phenomena have a similar chemical origin.

Since becoming aware that crude fuels contain phenols, we have traced back three old cases of colloid milium seen over the last 5 years which confirm the association noted by Holzberger (1960). These cases were white farmers who developed colloid milium on the hands, wrists, forearms and arms as far up as the short-sleeve line. The family history was negative and no other exposed area was affected. All had for years undertaken the repair of farm equipment (tractors, irrigation sprays) in full sunshine using 'Voco' (an inexpensive and crude power paraffin made by the Vacuum Oil Co.) to clean the equipment and their grease-soiled upper extremities. The picture was not quite the same as that of hydroquinone colloid milium with ochronosis, but the similarities are sufficiently interesting not to be dismissed as irrelevant at this stage.

An intriguing question lurks behind all observations of the foregoing type—what part do non-pigmenting phenols play in accelerating the ageing process everywhere in connective tissues under stress?

ACKNOWLEDGMENT

This work was supported by the South African Medical Research Council.

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Ultrastructural study of elaunin fibres in the secretory coil of human eccrine sweat glands

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Accepted for publication 7 March 1975

SUMMARY

The basement membrane of the secretory coil of human eccrine sweat glands was studied by light and electron microscopy. With classical staining methods for the elastic system, elaunin ring fibres were observed in the basement membrane of the secretory coil. The ultrastructural study showed that elaunin fibres are formed by bundles of fibrotubules 10–12 nm in diameter surrounding aggregates of amorphous material. These fibres differ from elastic fibres which are thicker and have an amorphous material more homogeneous and abundant.

In previous work, the distribution and morphology of the elastic system in the human dermis were studied and the existence of three types of fibres (oxytalan, elaunin and elastic fibres) was demonstrated (Bittencourt-Sampaio & Cotta-Pereira, 1971; Rodrigo, Cotta-Pereira & Riches, 1973; Cotta-Pereira, Rodrigo & Bittencourt-Sampaio, 1975). The most superficial are the oxytalan fibres; these depart from the elaunin plexus, which is located parallel to the dermo–epidermal junction and is connected with the elastic fibres of the reticular dermis.

The ultrastructure of elastic fibres is well known (Greenlee, Ross & Hartman, 1966; Ross & Bornstein, 1969). They consist of an amorphous substance identified as elastin surrounded by the 'elastic fibre microfibrils'. Recently, we observed that the oxytalan fibres are formed by bundles of fibrotubules similar to those visualized in elastic fibres, but devoid of elastin (Cotta-Pereira *et al.*, 1975). The fine structure of elaunin fibres is not yet known.

Observing sections of human dermis by light microscopy, we found elaunin fibres in the basement membrane† of the secretory coil of human eccrine sweat glands (Cotta-Pereira & Bittencourt-Sampaio,

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† In this paper the term 'basement membrane' is used to name the extracellular support of the basal surface of epithelia as observed by light microscopy. 'Basal lamina' is applied to the dense filamentous sheet visualized as a constituent of the basement membrane by electron microscopy.

1973). The purpose of this investigation was to study this area with the electron microscope in order to describe the fine structure of the elaunin fibres.

MATERIAL AND METHODS

Skin fragments biopsied from different areas of human volunteers were fixed in 10% formaldehyde and embedded in paraffin. Histological sections were stained by the following techniques: Verhoeff's iron haematoxylin, Weigert's resorcin fuchsin, Unna-Tanzer's orcein, and Gomori's aldehyde fuchsin. All these techniques were preceded or not by an oxidation with 40% peracetic acid (Pearse, 1968). The PAS technique after treatment with diastase was also used associated with the Weigert's resorcin fuchsin.

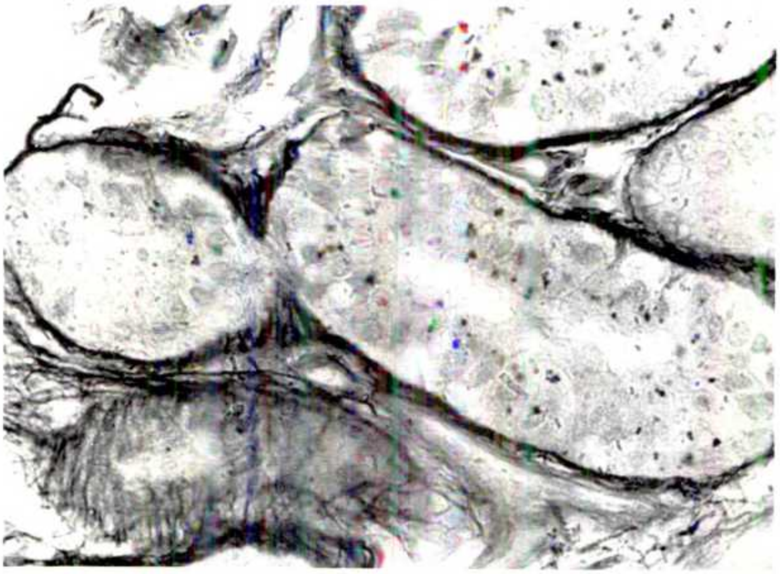

FIGURE 1. Elaunin fibres wrapping the secretory coil of a sweat gland. Weigert's resorcin fuchsin method (× 720).

Other fragments were fixed for 3 h in a mixture of tannic acid–glutaraldehyde (TAG), containing 0·25% tannic acid and 3% glutaraldehyde in Millonig buffer (pH = 7·4) (Futaesaku, Mizuhira & Nakamura, 1972). After postfixation in 2% osmium tetroxide they were embedded in Epon. The ultrathin sections were studied in a Siemens Elmiskop IA at 80 kV, after staining with uranyl acetate and lead citrate.

RESULTS

By light microscopy, using resorcin fuchsin, orcein or aldehyde fuchsin, an envelope of fibres was observed surrounding the sweat gland secretory coil (Fig. 1). When the resorcin fuchsin method was preceded by oxidation with peracetic acid, the fibrous structures were enhanced and thinner fibres intermingled with the thicker ones were visualized. After Verhoeff's stain, with or without previous oxidation, no fibres were observed (Fig. 2).

The association PAS-resorcin fuchsin showed a double stained membrane surrounding the secretory coils. While the diastase-resistant PAS-positive inner layer formed a continuous envelope around the

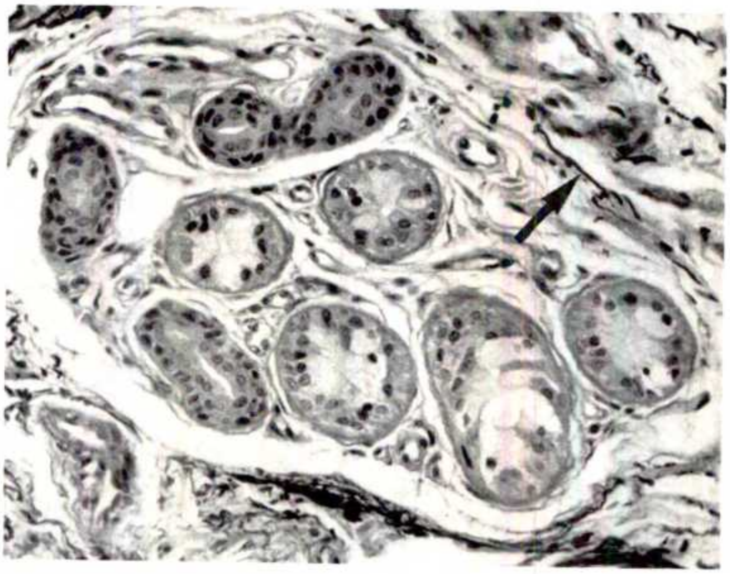

FIGURE 2. Secretory coil of a sweat gland. Elastic fibres are present only far from the gland (arrow). They are not seen in the secretory coil basement membrane. Verhoeff's iron haematoxylin (× 284).

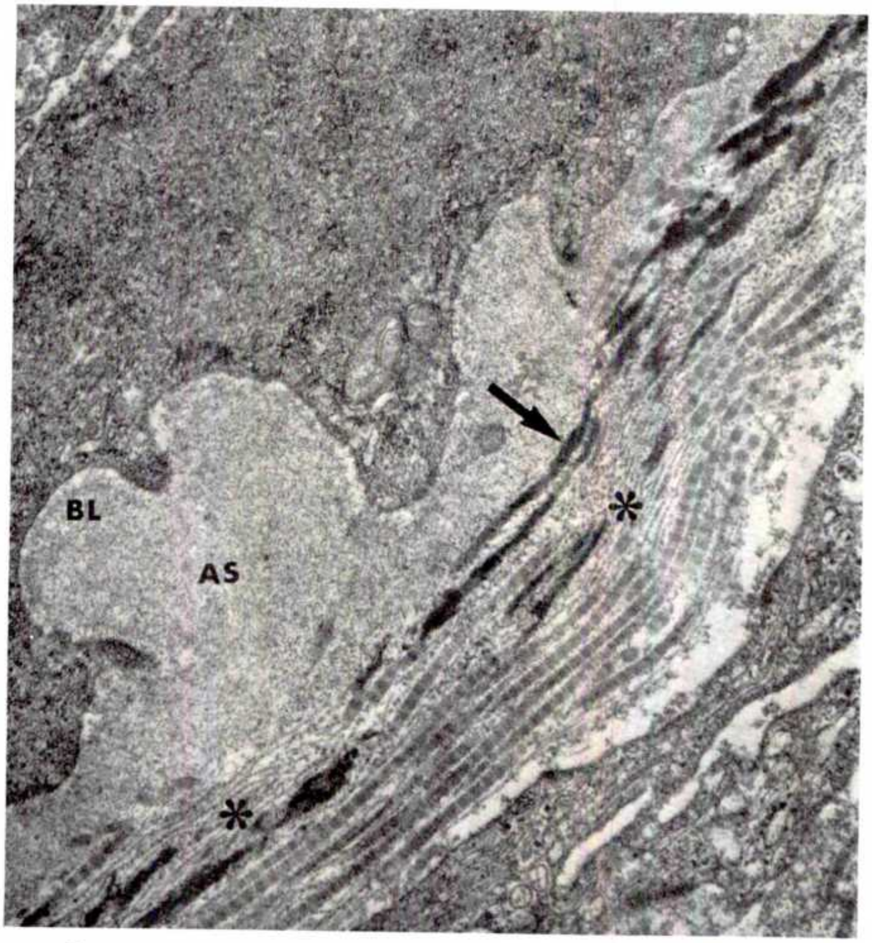

FIGURE 3. Basement membrane of a sweat gland secretory coil. Longitudinal section of an elaunin fibre, formed by patches of dense amorphous material (arrow) among fibrillar bundles (star). BL = basal lamina. AS = amorphous sheath (× 32,400).

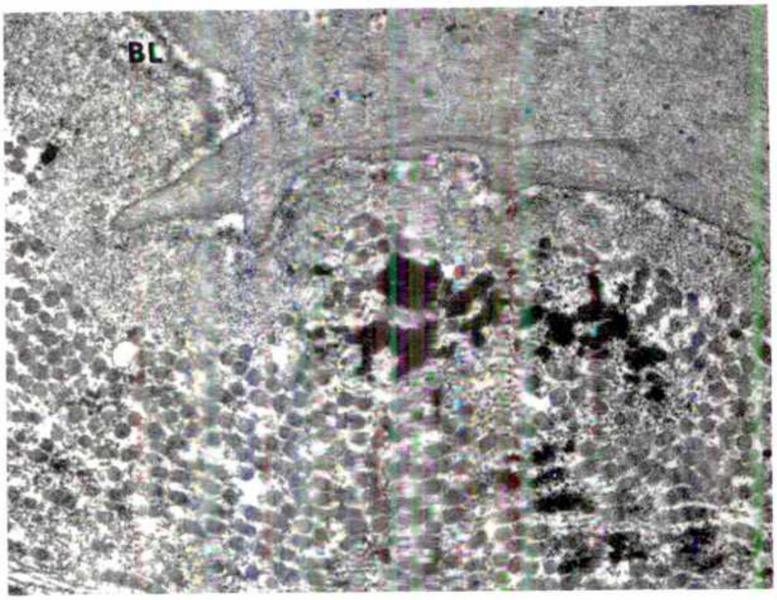

FIGURE 4. Basement membrane of a sweat gland secretory coil. Cross section of an elaunin fibre. Note the amorphous substance scattered among fibrotubular bundles (× 32,400).

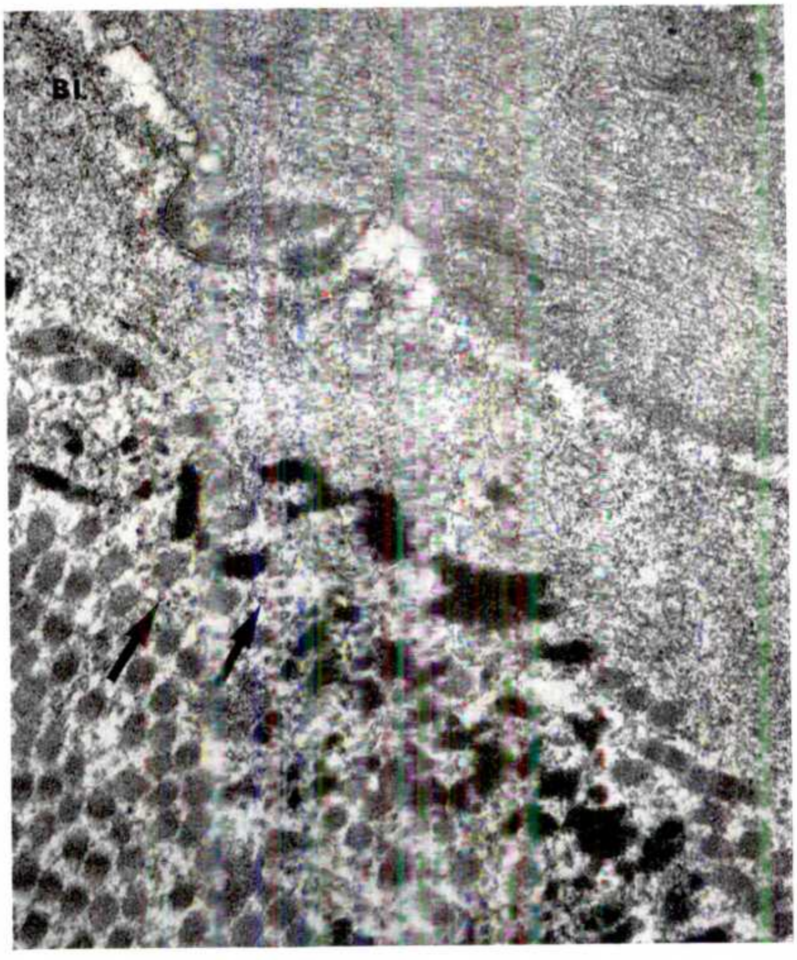

FIGURE 5. Basement membrane of a sweat gland secretory coil. Note the cross sectioned fibrotubules 10–12 nm in diameter (arrows) (× 64,800).

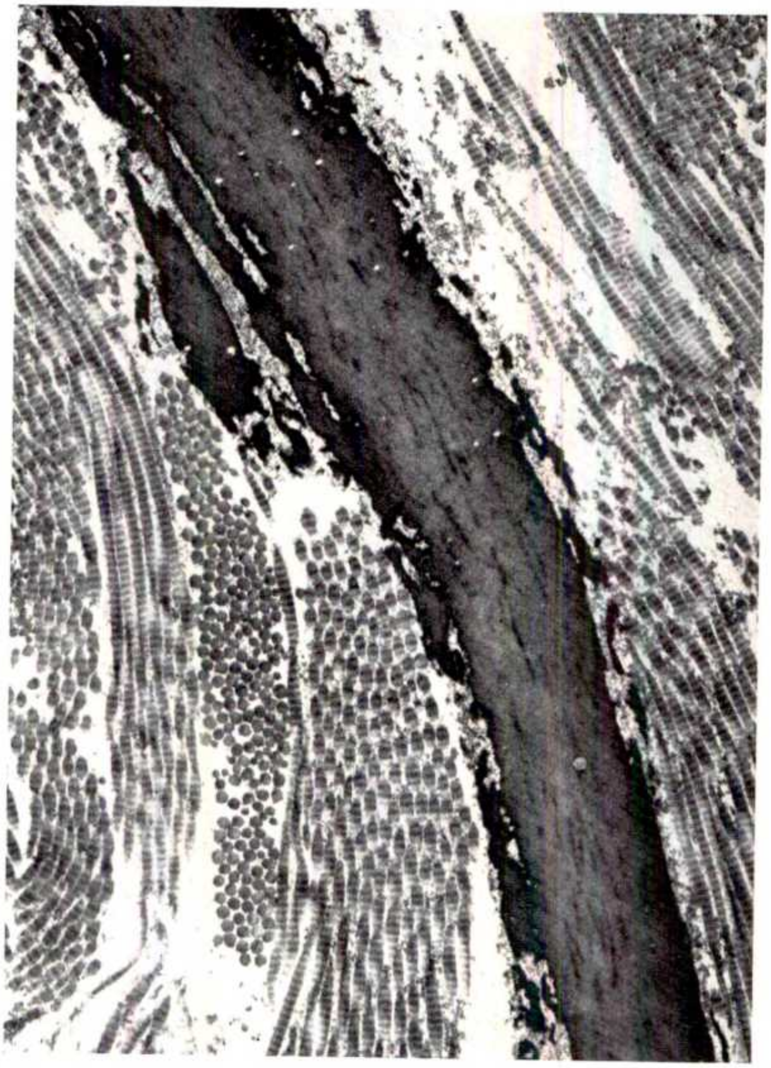

FIGURE 6. Longitudinal section of an elastic fibre. The amorphous component is compact and homogeneous. Some microfibrils are observed in the periphery of the fibre (× 16,200).

gland, the resorcin fuchsin stained outer layer showed different aspects. In cross sections, it often appeared as a discontinuous ring. In oblique or in longitudinal sections it was represented by a rank of points or parallel rods.

The electron microscopic study of the secretory coil basement membrane showed that it is formed of a basal lamina connected on its inner face with the serous and myoepithelial cells by half-desmosomes, and on its outer face with an amorphous and low electron dense sheath. This amorphous sheath has an irregular thickness and it is surrounded by collagen fibrils and by elastic-like fibres formed by patches of an electron dense amorphous substance in the core of fibrotubular bundles (Figs 3–5). This amorphous material is not so compact and homogeneous as it is in the elastic fibres (Fig. 6). Bundles of fibrotubules 10–12 nm in diameter and without amorphous material were also visualized.

DISCUSSION

Previously, it was demonstrated by light microscopy that the classical staining methods for elastic tissue could reveal in the human dermis oxytalan, elaunin and elastic fibres (Bittencourt-Sampaio & Cotta-Pereira, 1971; Cotta-Pereira *et al.*, 1975). Gawlik (1965) considered these three types of fibres as the normal constituents of the elastic system of connective tissues.

In the present work parallel ring fibres wrapping the secretory coil of sweat glands were demonstrated by resorcin fuchsin, orcein or aldehyde fuchsin but not by the Verhoeff's method. Fibres with similar tinctorial characteristics were revealed in tendons, fibrous and hyaline cartilage and elastic laminae of developing aortae (Gawlik, 1965). They were named 'elaunin fibres' by Gawlik (1965). On the basis of these tinctorial affinities, the sweat gland ring fibres are elaunin fibres, differing from elastic fibres because they do not stain with Verhoeff's method. Using resorcin fuchsin after oxidation with peracetic acid, thinner fibres binding the thick ring fibres were revealed. They have therefore the same tinctorial characteristics as the oxytalan fibres (Fullmer & Lillie, 1958; Gawlik, 1965). It is concluded that a mesh-work of elaunin and oxytalan fibres involves the secretory coil of eccrine sweat glands.

The main difficulty in studying elastic fibres with conventional electron microscopic techniques has been the irregular staining of their components. The introduction of tannic acid added to glutaraldehyde was a progress (Futaesaku *et al.*, 1972) simultaneously improving the fine details of the connective fibrils and giving a satisfactory staining of the elastin. With this method a good reproducibility of results is obtained. We tried several concentrations of tannic acid and the best results were observed with 0·25% added to 3% glutaraldehyde in Millonig's buffer.

With this technique, the electron microscopic study of the ring fibres in cross and in longitudinal sections showed that they are formed by bundles of parallel fibrotubules 10–12 nm in diameter surrounding aggregates of amorphous material. They differ from elastic fibres which are thicker and where the amorphous material is more homogeneous and abundant.

In his studies on elastogenesis, Gawlik (1965) found that in elastic laminae of developing aortae the oxytalan fibres appeared first and were later substituted by elaunin and finally by elastic fibres. Based on those observations oxytalan fibres may be considered as the most primitive elastic fibres. In agreement with this, are previous observations with the electron microscope demonstrating that oxytalan fibres are reduced to the fibrotubular component of the elastic fibres (Cotta-Pereira *et al.*, 1975). In fact, during elastogenesis the fibrotubular component is the first to appear and thereafter the accumulation of amorphous material is progressive until the mature elastic fibre is formed (Fahrenbach, Sandberg & Cleary, 1966; Greenlee *et al.*, 1966).

In light microscopy, the concept of the elaunin fibre is only based on tinctorial data. As the ring fibres of sweat glands have elaunin characteristics we studied them with the electron microscope in order to investigate their fine structure. We found that they have ultrastructural characteristics between the oxytalan and the elastic fibres. The fibrotubular bundles are abundant and the amorphous component is sparse.

Functionally, elaunin and oxytalan fibres have been related to mechanical resistance because they are found in places where mechanical stress is exercised (Fullmer, 1958; Gawlik, 1965). Perhaps their mechanical resistance is based on the fibrotubular component. This hypothesis is in accordance with the absence of elastin in oxytalan fibres and its scarcity in elaunin fibres. The function of the elaunin ring fibres in the sweat coil could represent a mechanical support for the gland, completed by the thinner oxytalan fibres linking the thick elaunin ring fibres.

Another fact to be discussed is the general structure of the basement membrane in the secretory coil of human eccrine sweat glands. From our observations, the inner layer, PAS-positive after diastase treatment, corresponds in electron microscopy to the area formed by the basal lamina and the amorphous sheath surrounding it. The outer layer is therefore constituted by elaunin, oxytalan and collagen fibres.

ACKNOWLEDGMENTS

Dr Cotta-Pereira was partly supported by the State Secretary of Science and Technology, Guanabara, Brazil. The authors wish to thank Dr J.F.David-Ferreira for his valuable discussion and criticism. Technical assistance was provided by Mr J.C.Lemos, Mrs Maria Teresa Plantier and Mrs Maria da Ressurreição Alpiorça. Miss Maria Manuela Magalhães typed the manuscript.

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British Journal of Dermatology (1975) **93**, 631.

The fine structure of the elastic tendons in the human *arrector pili* muscle

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Accepted for publication 14 March 1975

SUMMARY

The elastic tendons by which the *arrector pili* muscle is inserted into the hair follicle were studied by light and electron microscopy. From the follicle to the muscle it was observed that these tendons are formed successively by oxytalan, elaunin and elastic fibres. In close contact with the smooth muscle cells, elaunin and elastic fibres were observed. The fibres penetrate the spaces between the muscle cells and are clamped by them. An explanation is advanced about the significance of this arrangement in the recovery after muscle contraction.

The *arrector pili* muscle is implanted into the bulge of the follicular wall by means of elastic tendons (Nagel, 1945). The ability to analyse the organization of these structures by electron microscopy has recently been increased by the introduction of new methods for the study of the elastic fibres (Futaesaku, Mizuhira & Nakamura, 1972). As part of our study of the distribution and structure of the elastic system in human dermis (Cotta-Pereira, Rodrigo & Bittencourt-Sampaio, 1975 a,b) we have investigated the ultrastructural organization of the attachment of the *arrector pili* muscle to the hair follicle.

MATERIAL AND METHODS

Punch biopsies from hairy skin of normal human volunteers were fixed in 10% formaldehyde and embedded in paraffin. The specimens were sectioned at 5 μm and stained with Verhoeff's iron haematoxylin, Weigert's resorcin fuchsin, Unna-Taenzer's orcein and Gomori's aldehyde fuchsin. All these techniques were preceded or not by 40% peracetic acid oxidation (Pearse, 1968).

For the electron microscopic study some fragments were fixed for 3 h in a mixture of 3% glutaraldehyde and 0·25% tannic acid (Futaesaku *et al.*, 1972) in Millonig's buffer (pH = 7·4). After postfixation in 2% osmium tetroxide in the same buffer they were embedded in Epon. Thick sections stained with toluidine blue were used to select by light microscopy the blocks where hair insertions of the *arrector pili* muscle were present. Ultrathin sections from those blocks were studied in a Siemens Elmiskop IA at 80 kV, after staining with uranyl acetate and lead citrate.

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RESULTS

By light microscopy well developed fibres were seen linking the *arrector pili* muscle to the bulge of the hair follicle (elastic tendons). These fibres were revealed by all the staining methods used, but they were particularly enhanced when the Weigert's resorcin fuchsin technique was used after peracetic acid oxidation (Fig. 1).

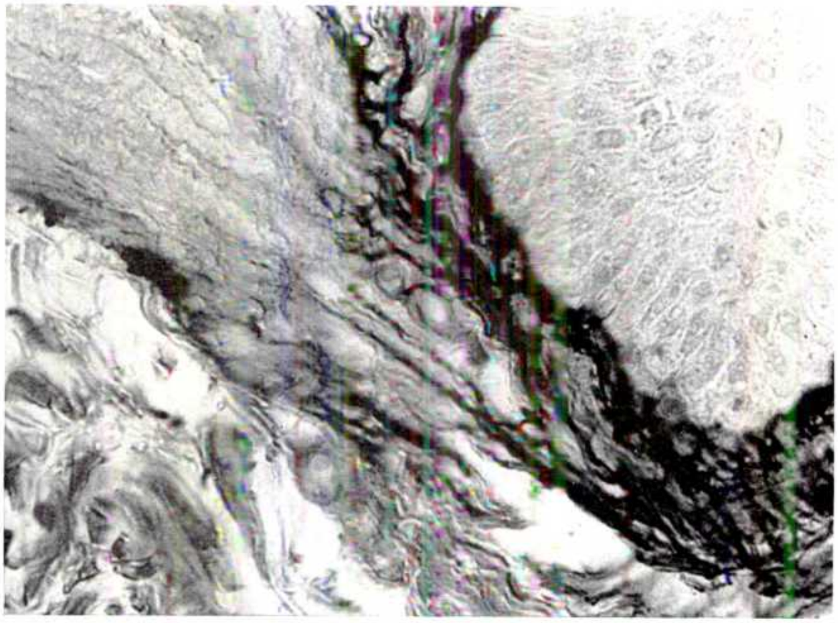

FIGURE 1. Hair insertion of the *arrector pili* muscle. Note the elastic tendons binding the extremity of the muscle to the bulge of the follicle. Weigert's resorcin fuchsin after peracetic acid oxidation (× 800).

In the electron microscopic study of the tendons it was observed that their anchorage to the hair follicle is made by short bundles of 10–12 nm diameter fibrotubules (Fig. 2). Similar bundles were previously identified on the basis of their stainability and ultrastructure as oxytalan fibres (Cotta-Pereira *et al.*, 1975a). Those bundles are in continuity with others, having in their core patches of an electron dense amorphous substance, which correspond, by the same criteria, to elaunin fibres (Cotta-Pereira *et al.*, 1975b). In other fibres the amorphous component, more compact and homogeneous, is predominant in relation to the fibrotubular one. These fibres are classified as fully mature elastic fibres. Elaunin and elastic fibres were found side by side, and they come into close contact with the extremity of the *arrector pili* muscle. In this area no isolated oxytalan fibres were found. The elaunin and the elastic fibres penetrate the space between the smooth muscle cells. Simultaneously

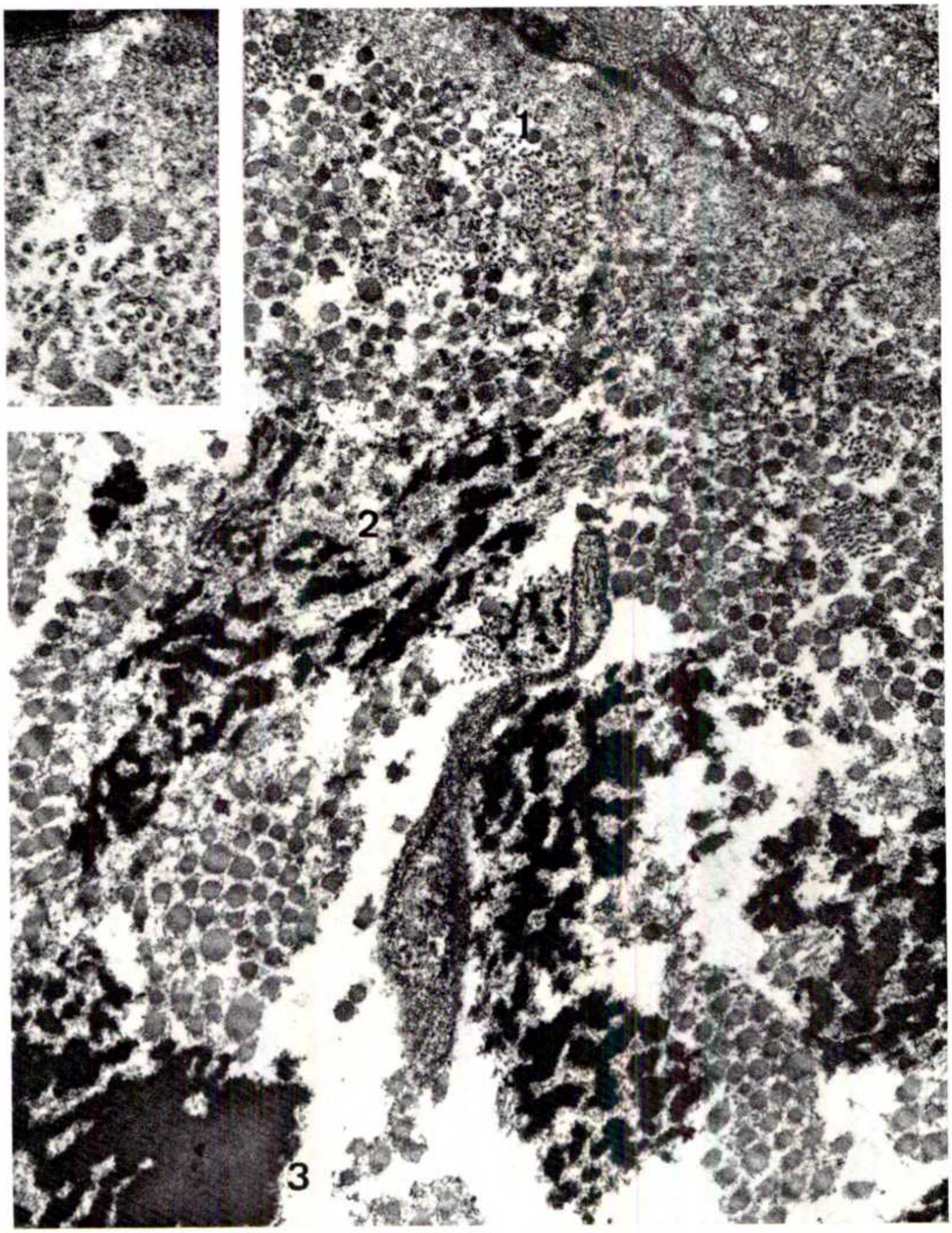

FIGURE 2. Hair insertion of the elastic tendons. The three types of fibres are observed in sequence: 1. Oxytalan fibre; 2. Elaunin fibre; 3. Elastic fibre (× 36,000). In the insert, higher magnification of a cross sectioned oxytalan fibre showing fibrotubules is seen (× 84,000).

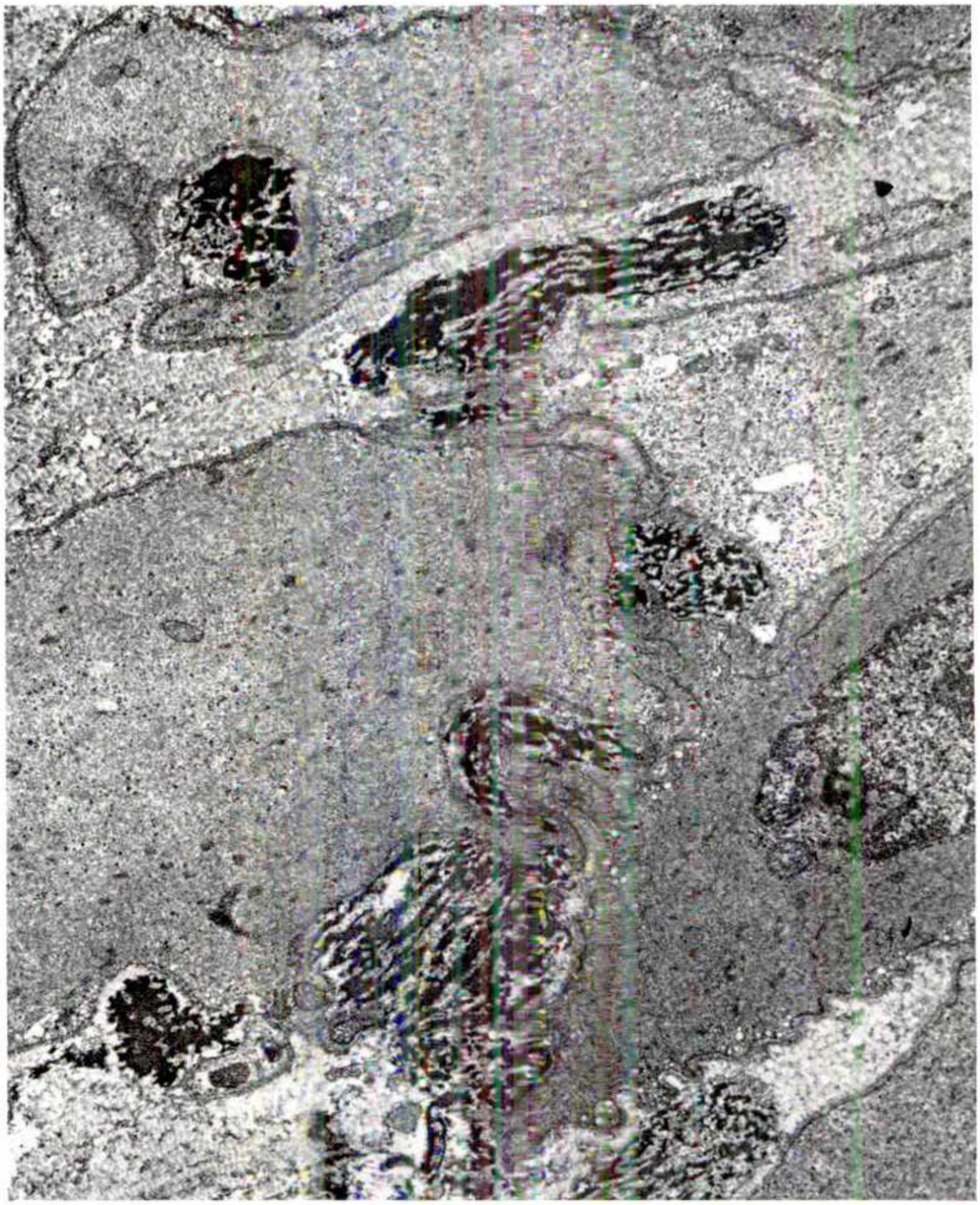

FIGURE 3. *Arrector pili* insertion of the elastic tendons. Elaunin and elastic fibres are seen among the smooth muscle cells. Note in the top of the figure a fibre clamped by a muscle cell (× 12,000).

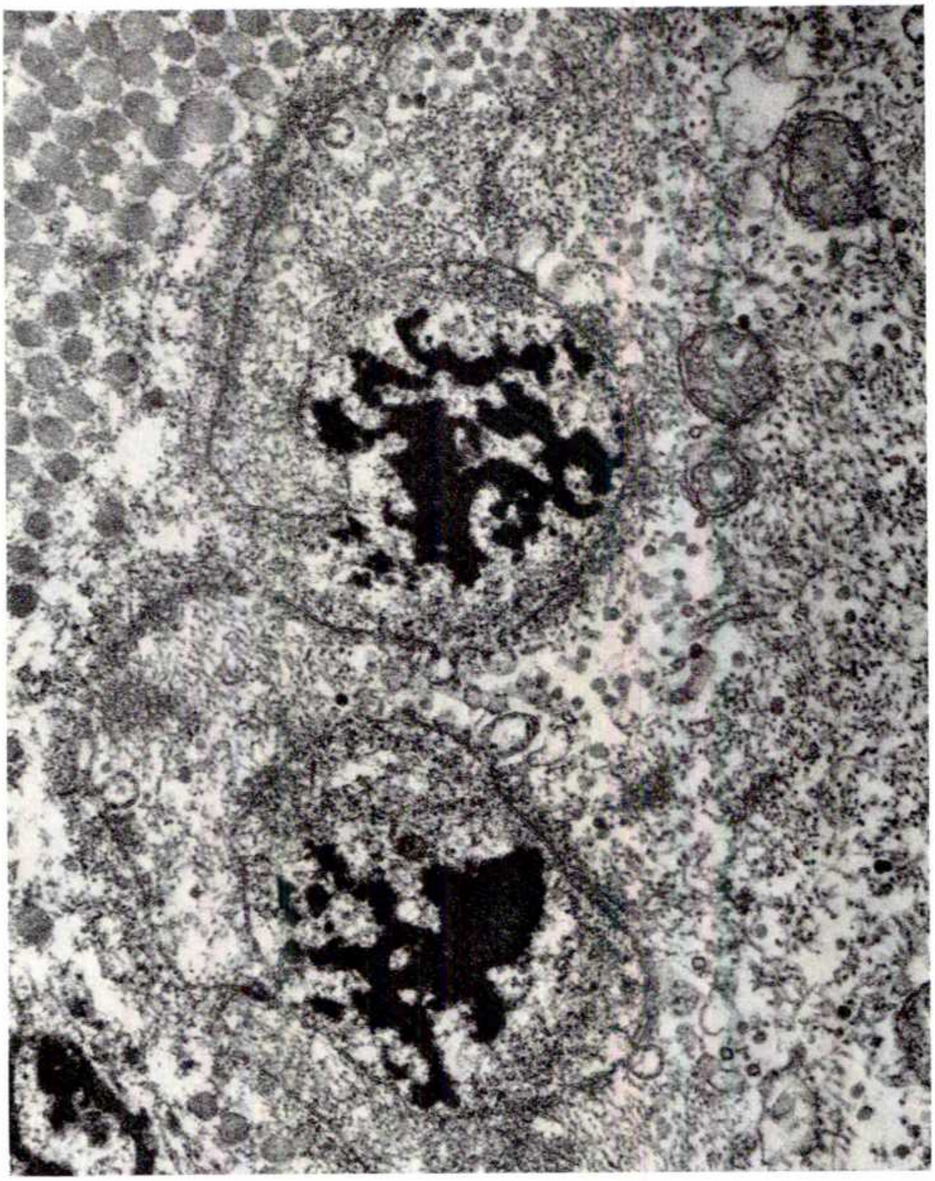

FIGURE 4. Two elaunin fibres are involved by processes of a smooth muscle cell. Note the close contact between the elastin and the cell membrane (× 60,000).

short smooth cellular processes are seen clamping the fibres and sometimes surrounding them (Figs 3 and 4).

DISCUSSION

The oxytalan, the elaunin and the elastic fibres, which are constituents of the elastic system (Gawlik, 1965), are recognized in light microscopy on the basis of their tinctorial affinities. The oxytalan fibres are stained with Weigert's method after peracetic oxidation (Fullmer & Lillie, 1958). The elaunin fibres are visualized after resorcin–fuchsin, orcein or aldehyde–fuchsin with or without previous oxidation, and are not seen after the Verhoeff's technique (Gawlik, 1965). The elastic fibres are revealed by all of these methods.

The association of tannic acid and glutaraldehyde during the specimen fixation is a good method for elastin staining in electron microscopy (Futaesaku *et al.*, 1972). With this technique the ultrastructure of the oxytalan and the elaunin fibres in the human dermis was studied in a previous work, and compared with the already known fine structure of the elastic fibres. The three types of fibres have well defined ultrastructural patterns. While the elaunin fibres are formed by scarce amorphous material intermingled with fibrotubular bundles, the oxytalan fibres are reduced to the fibrotubular component. The elastic and the elaunin fibres are only quantitatively different. The elastic fibres are thicker than the elaunin, and their amorphous component is compact and predominant (Cotta-Pereira *et al.*, 1975 a,b).

In studying by light microscopy the distribution of the elastic system in the dermis, we were impressed by the insertion of the *arrector pili* muscle into the bulge of the hair follicle, which is formed by fibres previously named elastic tendons (Nagel, 1945). These fibres were demonstrated by all of the staining methods used, with or without peracetic oxidation. According to the staining properties of the elastic system fibres, they could be formed by elastic fibres or by elastic fibres mixed with oxytalan and elaunin fibres. The ultrastructural study corroborates this second hypothesis. The three types of fibres were found in continuity from the follicle to the *arrector pili* muscle. The anchorage to the follicle is made by short oxytalan fibres, and the elaunin and the elastic fibres are clamped at the other side by the smooth muscle cells. As is shown in Figs 3 and 4, the arrangement between the fibres and the muscle suggests a firm mechanism of attachment.

Oxytalan and elaunin fibres are observed in areas where mechanical resistance is necessary, as in periodontium, ligaments, adventitia of blood vessels, epineurium, perineurium, tendons, and fibrous cartilage (Fullmer, 1958; Gawlik, 1965). This fact suggests that the anchorage system between the *arrector pili* muscle and the hair follicle is well adapted to a biphasic mechanism of contraction and distension. Thus the recovery to the rest position may be based on the tensive and the elastic properties of the follicular insertion.

ACKNOWLEDGMENTS

Dr Cotta-Pereira was partly supported by the State Secretary of Science and Technology, Guanabara, Brazil. The authors wish to thank Mr J.C.Lemos, Mrs Maria Teresa Plantier and Mrs Maria da Ressurreição Alpiarça for their technical assistance, and Miss Maria Manuela Magalhães for typing the manuscript.

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British Journal of Dermatology (1975) **93**, 639.

The influence of age and sex on skin thickness, skin collagen and density

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Accepted for publication 1 April 1975

SUMMARY

Forearm skin collagen, dermal thickness and collagen density were measured in a large number of normal subjects as a standard reference for future studies. Skin collagen decreased with age and was less in the females at all ages. There is a direct relationship between skin collagen and dermal thickness but variations in collagen density in disease limit the use of dermal thickness as a guide to changes in its collagen content.

In the course of studies on the factors controlling skin collagen content in disease a large number of observations have been made on normal subjects. By measuring skin thickness at the same time we were also able to calculate collagen density (Black, Bottoms & Shuster, 1970; Shuster, Black & Bottoms, 1970; Black, Bottoms & Shuster, 1972 a, b & c, 1973). These measurements define certain of the factors controlling normal dermal thickness and collagen content and provide data which can serve as a reference point for future studies.

MATERIALS AND METHODS

Skin Collagen

Skin collagen was measured in seventy-four Caucasian males and eighty females aged 15–93 years. Most were attending the Department of Dermatology with a variety of minor localized skin disorders. A few of the skin samples were taken at post mortem. None of the patients was known to be suffering from endocrine or chronic wasting disease, nor had any been given corticosteroids.

The method was that of Shuster & Bottoms (1963). Biopsies were taken from the mid-point of the extensor aspect of the forearm using a high-speed 5 mm punch. In a few instances skin was taken in the same way at post mortem, since we have shown this to be without effect on total skin collagen content (unpublished observations). The biopsies and post mortem samples were defatted in acetone, dried to constant weight, hydrolysed and their hydroxyproline content measured by the method of Woessner (1961). The collagen content was then expressed as a function of the surface area of the biopsy since this is the most satisfactory way to measure skin collagen (Shuster & Bottoms, 1963; Shuster, Raffle & Bottoms, 1967 a, b).

Skin thickness

Skin thickness was measured in ninety Caucasian males and 107 females of age 12–93 years. The method was that of Meema, Sheppard & Rapoport (1964) as modified by Black (1969).

Collagen density

In twenty-seven males and twenty-six females both skin thickness and skin collagen were measured on adjacent areas of forearm skin and the collagen density was calculated from this.

RESULTS

1. *Skin collagen*

The relationship of skin collagen to age and sex is shown in Fig. 1(a and b). There is a linear decrease in skin collagen with age and the male forearm skin contains more collagen than the female at all ages studied.

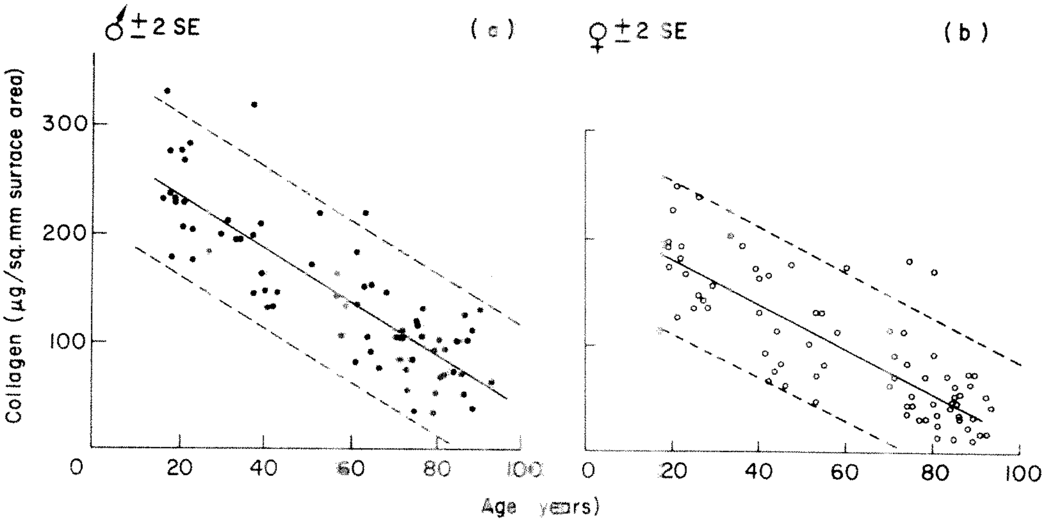

FIGURE 1. The relationship of skin collagen content (expressed per unit skin surface area) to age in males (a) and females (b). Females have less collagen than males at all ages but the rate of decrease is the same in both sexes.

2. *Skin thickness*

The relationship of skin thickness to age and sex is shown in Fig. 2(a and b). For males at all ages studied a highly significant regression ($P<0\cdot001$) was found showing gradual thinning of the skin with advancing age. By contrast in the female, skin thickness remained constant up until the 5th decade after which there was a significant decrease with age ($P<0\cdot001$).

3. *Relationship of skin collagen to thickness*

There was a highly significant relationship ($P<0\cdot001$) between thickness and the skin collagen content (Fig. 3a) for males at all ages studied. A similar and equally significant relationship ($P<0\cdot001$) was found for females over 60 years of age but not in females under 60 years of age (Fig. 3b).

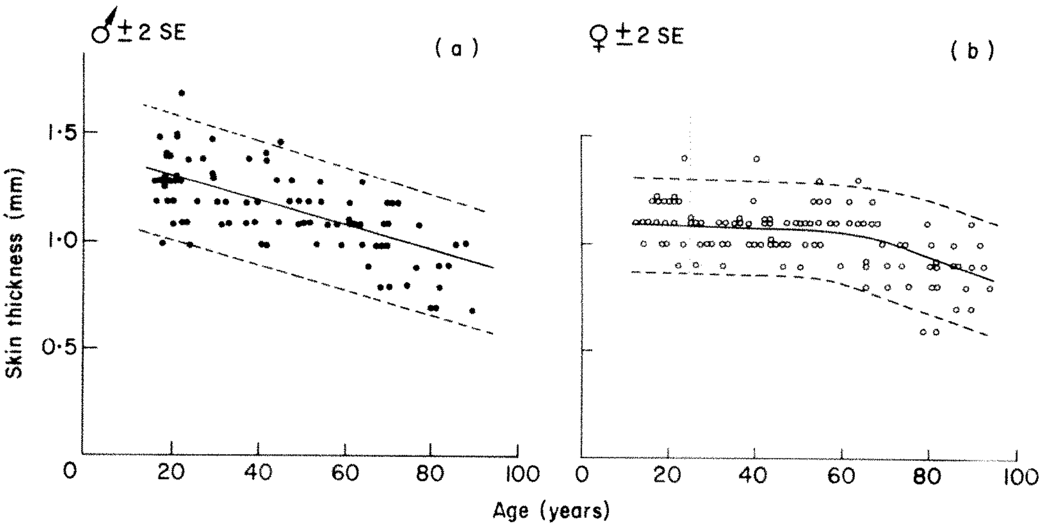

FIGURE 2. The relationship of skin thickness to age in males (a) and females (b). A relationship is apparent at all ages for males but only after middle age for the females.

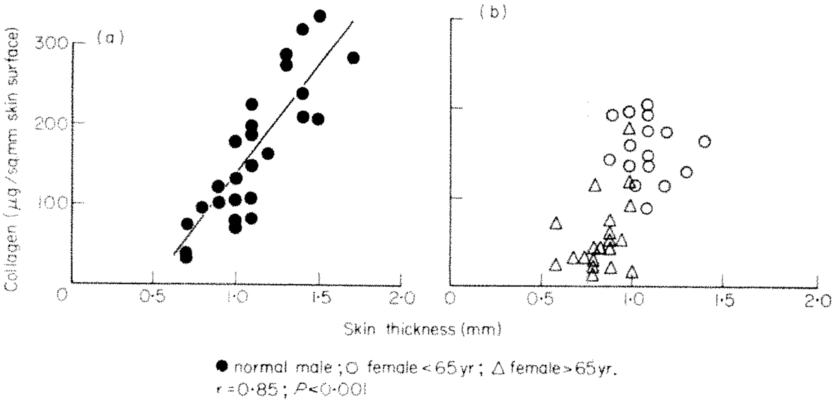

FIGURE 3. The relationship of skin collagen and skin thickness in males (a) and females (b).

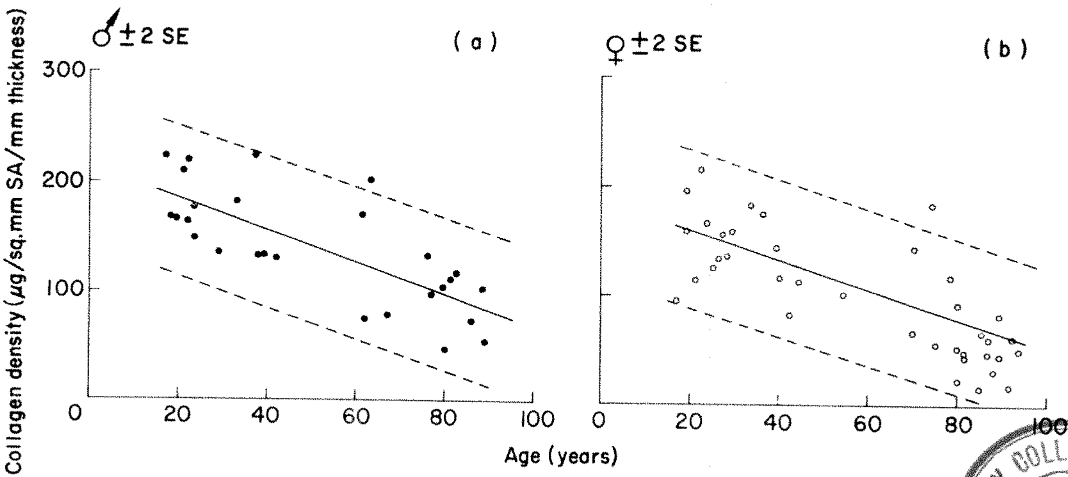

FIGURE 4. The relationship of skin collagen and density to age in males (a) and females (b).

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4. *Collagen density*

Collagen density calculated as the ratio of skin collagen to thickness was very significantly ($P<0{\cdot}001$) related to age in both males and females (Fig. 4a and b) but the density was consistently lower in females at all ages.

DISCUSSION

The present observations provide values for skin collagen content, density and skin thickness in relationship to age in normal men and women. So far as skin collagen is concerned the clear relationship to age is apparent, skin collagen decreasing linearly by about 1% per year throughout adult life. Failure of previous investigations to find this decrease is due to the artefact of expressing skin collagen as a percentage, or in relationship to some other constituent of skin (Elden, 1970; Shuster & Bottoms 1963; Black *et al.*, 1970a). This difficulty is avoided if parameters of skin structure and function are referred to surface area (Shuster & Bottoms, 1963; Shuster *et al.*, 1967a, 1967b; Black *et al.*, 1970a). The rate of loss is the same in both males and females although total skin collagen content is less in females than males at all ages. One reason for this sex difference in skin collagen content may be androgen (Shuster *et al.*, 1970; Black *et al.*, 1970b; Burton *et al.*, 1972). In adult skin the clinical features of ageing are closely related to the total collagen content (Shuster & Bottoms, 1963). The lower initial skin collagen content is therefore the reason women appear to age earlier than men. So far as their skin is concerned, age for age women are about 15 years older than men throughout their adult life. Although we have presented data from extensor skin of the forearm we have found precisely the same to be true of skin all over the body whether sun exposed or protected; moreover there is a direct correlation between the collagen content of skin taken from different parts of the body in all individuals studied; so that skin collagen content on the forearm can be taken as representative of all skin sites (Shuster & Bottoms, unpublished observations).

The finding of age and sex related changes in skin thickness was expected on clinical grounds (Shuster & Bottoms, 1963) and these changes are gross enough to be taken into account in studies of endocrine and other diseases (Stevenson, Bottoms & Shuster, 1970; Black *et al.*, 1970a,b; Shuster *et al.*, 1970; Black *et al.*, 1972a, 1972b, 1972c, 1973; Meema *et al.*, 1964). The constancy of skin thickness in adult women up to the 5th decade did surprise us and is not in agreement with the findings of Meema *et al.* (1964) who found a decrease at all ages. Although our modification (Black, 1969) of the method of Meema *et al.* (1964) reduces the effect of rotation of the arm and focus of the X-ray beam, the difference seems to be too great for such a methodological explanation. This part of our study would be worth repeating because the dissociation of skin thickness and collagen content up to the 5th decade in females implies either the structural maintenance of a looser dermal architecture or a greater density of ground substance between the collagen bundles as the primary event.

As with collagen and thickness, collagen density the packing of fibrils in the dermis, is also influenced by age and sex. With increasing age, skin collagen decreases more rapidly than skin thickness so that collagen density decreases. That skin collagen is less densely packed in the female than in the male may be due to androgen since skin collagen density is increased in patients with primary cutaneous virilism (Shuster, 1972). Our findings also confirm that collagen is a major component of skin thickness; with the exception of the younger female skin, age related changes in thickness correlate well with skin collagen content. We have also shown this by a direct comparison of skin collagen measured chemically, and thickness measured histologically (Bottoms & Shuster, unpublished). Since however skin collagen density is also affected by other metabolic factors and by disease (Black *et al.*, 1972b, 1972c, 1973, 1970) skin thickness cannot by itself be used as a measure of skin collagen content.

The measurement of skin thickness, collagen content and density has provided useful information in metabolic and endocrine diseases; it may also prove useful in monitoring the effect of drugs acting on connective tissue and in the investigation of diseases in which a generalized defect of connective tissue is suspected. Our present data in normal individuals are sufficient in number to use as a reference point for such future studies.

ACKNOWLEDGMENT

We are grateful to Wellcome Trust for a grant.

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British Journal of Dermatology (1975) **93**, 645.

Palmar ridge appearances in normal newborn infants and ridge appearance in relation to eccrine sweating

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Accepted for publication 14 March 1975

SUMMARY

Epidermal ridge appearances over the palm in twenty normal newborn infants are described. No clear-cut relationship between ridge appearance and sweating was found although gross ridge flattening was always associated with a negative *o*-phthaldialdehyde sweat test.

Epidermal ridges are differentiated during the 3rd and 4th fetal months, attaining their definitive morphology and changing thereafter only by increase in size (Cummins, 1971). No new sweat glands are formed after birth and the density of eccrine sweat glands in a particular area changes in inverse relation to the growth of the area.

In this study palmar ridge appearances were observed in normal newborn infants. In addition, ridge appearances were considered in relation to the absence or presence of eccrine sweating.

PATIENTS AND METHODS

During the course of a study on palmar sweating in ninety-one newborn infants (Verbov & Baxter, 1974), plastic imprints of skin (Sarkany, 1962) were taken in twenty of these babies (11 males, 9 females). In this method liquid silicone rubber monomer (Silflo) is mixed with a few drops of catalyst and applied to the skin. The plastic hardens in 3–4 min and is then gently lifted away; this provides a replica of the skin appearance which can be photographed. The area imprinted was the left hypothenar area just proximal to the distal transverse flexion crease. Imprints were taken during the first 6 days of life, and the youngest child imprinted was 4 h old.

Palmar epidermal ridge appearances as seen on imprints were considered in relation to an *o*-phthaldialdehyde (OPT) sweat test (Juhlin & Shelley, 1967), performed without any form of stimulation, over a small area of the right hypothenar region (Verbov & Baxter, 1974) at the same time as the imprinting.

RESULTS

Palmar ridge appearances were variable, with some flattening visible in all infants and ridge breadth

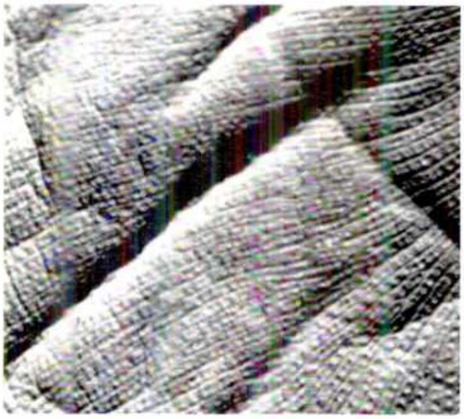

FIGURE 1. Male infant aged 10 h. Right hypothenar sweat test was positive.

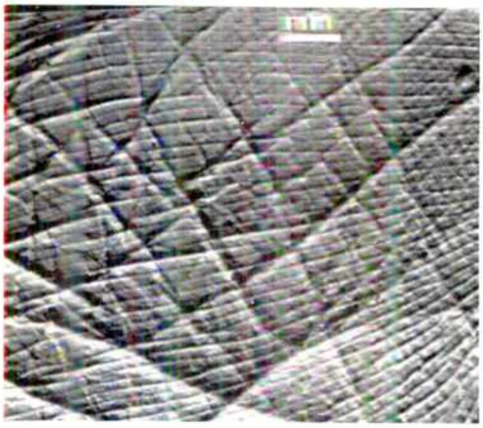

FIGURE 2. Male infant aged 3 days; dry palm. Right hypothenar sweat test was negative.

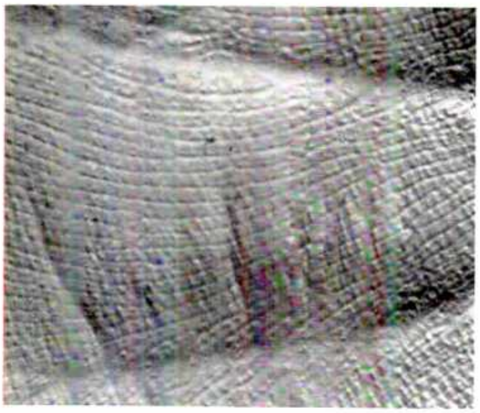

FIGURE 3. Female infant aged 11 h. Note broad and more flattened ridges compared to Figs 1 and 2. Right hypothenar sweat test was positive.

All the illustrations are of left hypothenar imprints from normal newborn infants (× 8).

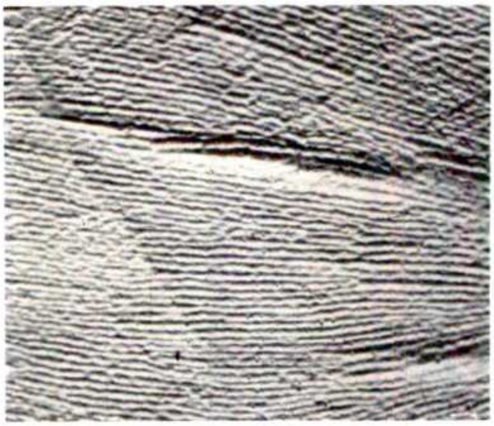

FIGURE 4. Male infant aged 3 days. Note narrow ridges. Right hypothenar sweat test was positive.

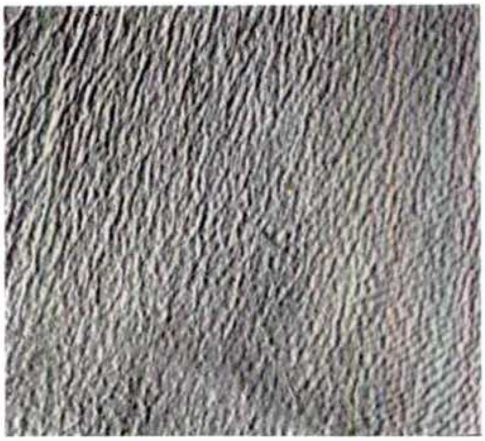

FIGURE 5. Female infant aged 21 h. Note incompletely differentiated ridges. Right hypothenar sweat test was positive.

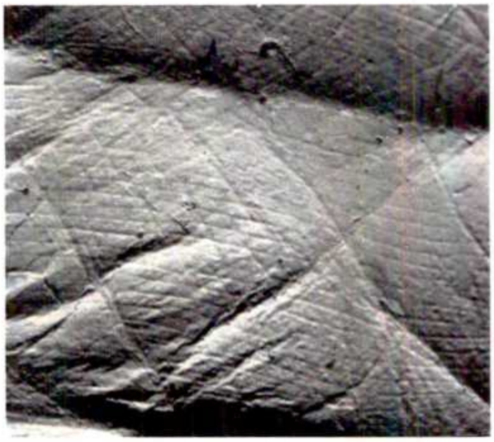

FIGURE 6. Male infant aged 8 h. Marked ridge flattening; dry palm. Right hypothenar sweat test was negative.

showing a wide range (Figs 1–4). Ridges were clearly distinguishable from adjacent valleys. An incompletely differentiated appearance of ridges (Fig. 5) was seen in some areas of the imprint in five infants but four of these infants demonstrated sweating.

Although there was no clear-cut relationship between eccrine sweating (as detected by the OPT test) and ridge height, marked ridge flattening (Fig. 6), present in four infants, was always associated with a negative sweat test. The older an infant was imprinted the more likely was ridge flattening to be minor and sweating to be present: in fact, three of the four infants with marked ridge flattening were tested in the first 14 h of life.

DISCUSSION

The hypothenar palmar ridge appearances found, apart from incomplete ridge differentiation, are seen in individuals of any age. But whereas in older individuals flattening may have many causes, in normal babies it is more likely due to the immature water-holding capacity of the stratum corneum together with incomplete function of the eccrine sweat unit. Thus, the consistent finding of ridge flattening in the imprints is in keeping with the age of the individuals tested because it is with both normal growth and sweat gland function that ridge elevation becomes more prominent.

ACKNOWLEDGMENTS

I should like to thank Professor A.D.Hay for permission to test the babies, and Miss J.Dorrington of the Department of Medical Illustration, St. Bartholomew's Hospital, London, for the illustrations.

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British Journal of Dermatology (1975) **93**, 649.

Epidermal transit times

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Accepted for publication 20 March 1975

SUMMARY

Radio-isotope disappearance curves and autoradiography (labelled DNA precursors and labelled amino acids) have been used to estimate the minimum transit times for four regions of epidermis in mice. The post-mitotic maturation time in the basal layer has been estimated and the results interpreted in relation to proliferation rates and epidermal organisation.

The time taken for a cell to pass from the basal layer to the superficial cornified layer, the transit time, can be measured by marking basal cells and sampling the tissue as the marked cells pass through the layers; the time taken for the first marked cells to appear at the surface being recorded. Unfortunately this technique is complicated by a number of factors: (1) it is semi-quantitative, (2) the cornified cells in epidermis are thin dead cells which are difficult to identify individually, (3) it is not clear how long the marked basal cells remain in the basal layer before moving into the superficial differentiating layers, (4) the material used for marking the cells may be released and re-utilized during transit through the layers, and (5) it records the minimum, not the average transit time. Radioactive markers can be measured quantitatively but points (2)–(5) still apply. Counting the radioactivity in thin strips of cornified cells removed from the skin surface will indicate the time taken for the radioactivity to appear in the superficial layers but it is difficult to control the number of cell layers being removed and counted.

In this report an attempt has been made to combine the quantitative aspects of radio-isotope counting with the qualitative aspects of autoradiographic observations (both techniques having their limitations) to determine the total time taken for cells to pass through epidermal tissue of differing thickness from various sites in the mouse and to interpret the results in relation to rates of epidermal proliferation.

MATERIALS AND METHODS

Male DBA-2 mice between the ages of 7 and 8 weeks were used. Mice of this age weigh about 20 g and have dorsal skin in the resting phase of hair growth (Silver, Chase & Arsenault, 1969). Samples of full thickness skin were taken from the mid-dorsum, ear, tail and plantar surface of the foot.

The following isotopes were used: ^{3}H (methyl) thymidine (^{3}HTdR) (5 Ci/mM) 50 μCi per mouse, ^{125}Iododeoxyuridine (^{125}IUdR) (1 μCi/μg) 75 μCi per mouse, ^{3}H (methyl) methionine (8·2 Ci/mM)

50 μCi per mouse and ^{35}S-cystine (87 mCi/mM) 20 μCi per mouse. All the isotopes were obtained from the Radiochemical Centre, Amersham, England and were injected intraperitoneally at various times (up to 35 days) before killing the animal and removing tissue samples. The tritium and ^{35}S labelled material was counted in a Packard Liquid scintillation counter (model 3375) at 5°C with the counting window width set at 115–1000 with 50% amplification. This setting minimized chemiluminescence in samples dissolved in Soluene (Packard Instrument Company Ltd.). The ^{125}I was counted in a Gammaguard machine (Tracerlab Ltd.).

The radioactivity was measured either in fixed (Carnoy's fixative followed by storage in 70% ethanol) full thickness skin or in samples where adhesive tape had been used to strip the superficial layers. The fixation of DNA precursor labelled material removed essentially all non-DNA bound radioactivity (Potten, 1973). The autoradiographs of the amino acid labelled samples indicated that fixation removed sufficient non-protein-bound material to show some specificity of label distribution in the epidermis.

The full thickness samples contain hair follicles, connective tissue, muscle and fat, some of which take up label (Hamilton & Potten, 1972; Potten, Jessup & Croxson, 1971). However, the hair follicle, muscle and fat activity has a minimal turnover during the time intervals studied. The hair follicles are in the dormant phase of the hair growth cycle (Silver *et al.*, 1969; Hamilton & Potten, 1972) while the epidermis is under constant renewal. Thus DNA bound activity will 'turn over' at a rate related to the cellular turnover and epidermal thickness. The techniques for plucking the hairs, and stripping with tape have been described elsewhere (Pinkus, 1951; Hennings & Elgjo, 1970; Potten, 1968, 1972). After the animals were killed the entire dorsal skin was removed and the hair was clipped. Half the skin was spread flat on a cork board and a 3 mm dermal punch was used to remove numerous circular full thickness skin samples which were subsequently fixed. The other half was exposed to a commercial depilatory agent to remove the hair stubble after which clear adhesive tape was applied and removed and 6 mm diameter circular punches of the tape were taken. Three tape punches were placed together in a scintillation vial and counted. The results were expressed as either counts or disintegrations per mg (foot) or per surface area (7·1 mm^2) (other full thickness epidermal regions), or per surface area (85 mm^2) (tape strippings).

Fixed samples of all tissues were also taken for sectioning (6–7 μm) and preparation for autoradiography (Ilford K5 emulsion 3 weeks exposure).

A total of 292 mice were used for the ^{3}HTdR experiment, 160 mice for the ^{125}IUdR, 120 mice for the ^{3}H-methionine and 153 mice for the ^{35}S-cystine experiments. These numbers of animals provided whole skin samples for most of the four epidermal regions studied. Tape punch samples were taken only from dorsal skin.

The rationale for the experiments was as follows: (1) ^{3}HTdR and ^{125}IUdR are both incorporated into new DNA at the time of its synthesis. This would occur only in nuclei of the proliferative cells which in mouse dorsal and ear epidermis are restricted to the basal layer. Once off the basal layer the cells arrange themselves into columns and flatten to cover an area 10 times that of a basal cell and equal to that of a cornified cell (MacKenzie, 1969; Christophers, 1971; Potten, 1974; Allen & Potten, 1974; Potten & Allen, 1975; Christophers, Wolff & Laurence, 1974). These columns and the ten proliferative basal cells can be regarded as discrete units of proliferation, the Epidermal Proliferative Unit (EPU) (Allen & Potten, 1974; Potten, 1974), each unit producing about one cell per day in dorsum and ear skin (unpublished observations; Potten, 1974; Allen & Potten, 1974; Potten & Allen, 1975). It normally takes a few days for label to appear in the first suprabasal layer (Christophers *et al.*, 1974; Iversen, Bjerknes & Devik, 1968) which indicates that there are a few cells in the basal layer that have finished their division phase and are maturing before migrating (post-mitotic maturing cells). Once off the basal layer the cells 'flow' steadily towards the surface at a rate per layer equivalent to the cell production rate in the basal region of the unit. Thus the total transit time will be related to the basal cell production rate and the number of suprabasal cell layers. When a cell reaches the granular layer the nucleus is degraded and much of the nuclear labelled material is released and some re-utilized. This would result in a fall in total radioactivity levels after a time equivalent to the transit time from basal layer to top granular layer. Any radioactivity not released and re-utilized or catabolized might become trapped in the cornified cells to be released when these cells reach the surface. Such radioactivity might be detectable in tape punches. (2) Labelled amino acids would be incorporated into cells in both the basal layer and the living differentiating layers (spinous and granular layers). This label would become incorporated into the cornified cells when they passed through the cornification process and would then be carried to the surface to appear in the tape punches. As with ^{3}HTdR some radioactivity may be recycled. When these labelled cells reach the surface the total radioactivity levels would begin to fall. The nuclear label (total radioactivity measurements) would thus provide an estimate for the basal to top granular cell transit time which would include the postmitotic maturation time while the cytoplasmic (amino acid) label would measure the transit from top granular to the surface. The tape punch results would provide an estimate for the transit from basal to surface, including postmitotic basal maturation time (nuclear label) and the transit from granular to surface (amino acid label). Combination of all the data after correction for basal maturation time would give the true total transit time.

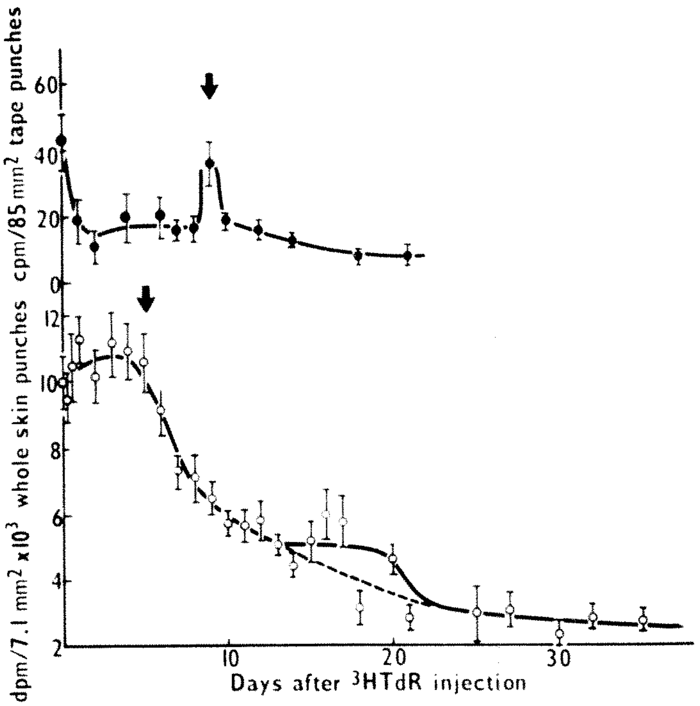

FIGURE 1. The radioactivity disappearance results (dpm) (lower) and the radioactivity in tape punch results (cpm) (upper) for dorsal skin are plotted against the time after administration of tritiated thymidine. The arrows mark the main points of reference for the transit experiments. Each point represents the mean and standard error of between 4 and 23 animals. The tape punch experiment used 132 mice and the skin punch experiment 292 mice. The results for several experiments have been pooled.

TABLE 1. Epidermal transit measurements (days)

	Dorsal skin		Ear		Tail		Foot	
	Cell layers	Transit	Cell layers	Transit	Cell layers	Transit	Cell layers	Transit
(A) Basal to top granular	3	5* 5†	3·5	5* 5†	5·5	5* 6–7†	11	5* 4†
Mean		5 (1·67)		5 (1·43)		6 (1·09)		4·5 (0·41)
(B) Top granular to surface	6	5‡ 7‡¶ 5§¶	11	8‡ 9§	32	7‡ 9§	30	3‡ 7§
Mean		6 (1·00)		8·5 (0·77)		8 (0·25)		5 (0·17)
(C) Basal to surface	9	9*¶ 12†¶ 12†	14·5	17* 12†	37·5	12*	41	—
Mean		11 (1·22)		14·5 (1·00)		12 (0·32)		—
Total transit A+B	9	11 (1·22)	14·5	13·5 (0·93)	37·5	14 (0·37)	41	9·5 (0·23)

* ^{3}HTdR liquid scintillation counting; † ^{125}IUdR liquid scintillation counting; ‡ ^{3}H-methionine liquid scintillation counting; § ^{35}S-cystine liquid scintillation counting; ¶ tape strip measurements (1+ layers removed with tape)

The measurement of transit from basal to top granular layer (^{3}HTdR or ^{125}IUdR) includes the post-mitotic basal layer maturation time. The ^{3}HTdR, ^{125}IUdR and ^{3}H-methionine results were supported by autoradiographic observations. Figures in brackets are the average time spent in each cell layer.

The number of cell layers in the epidermis was determined by counting the number of nucleated and cornified cell layers at 40 random points on either 6 μm paraffin embedded sections or 1 μm epon-araldite embedded material.

RESULTS

Fig. 1 shows a typical set of radioactivity changes at various times after precursor injection; in this case for ^{3}HTdR and dorsal skin. The radioactivity levels in fixed skin remain fairly constant for a period of about 5 days and then begin to fall. The radioactivity levels in the tape punches show a small peak on the 9th day. Fig. 2 shows a typical set of data for labelled amino acids; in this case for ^{3}H-methionine and dorsal skin. The total radioactivity values show considerable scatter but suggest fairly constant levels for about 5 days followed by a fall, while the tape punches (^{35}S-cystine, dorsal skin) tend to show a sharp rise on the 5th day, followed by a series of fluctuations at the new elevated level. The data from all the experiments are summarized in Table 1. The estimates for the total transit times (including basal maturation times) are shown for the four regions of skin.

Figs 3 and 4 show autoradiographic data supporting the liquid scintillation counting results. Four days after exposure to ^{3}HTdR or ^{125}IUdR, label is visible in the upper granular cells in ear and foot and is visible in the third or fourth layer in tail samples (Fig. 3). Dorsal skin (autoradiograph not shown) also has label in some upper granular cells on the fourth day. Some ^{3}HTdR or ^{125}IUdR label can be observed to be trapped in the cornified cells after nuclear breakdown in the upper granular cell (i.e. about 6–8 days after injection).

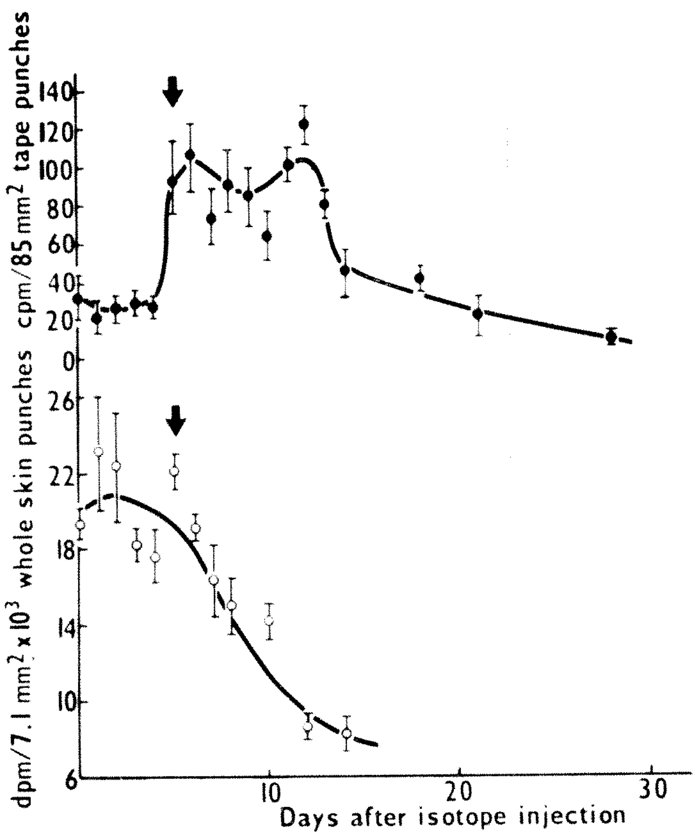

FIGURE 2. The radioactivity disappearance results (dpm) (lower) for tritiated methionine, and the radioactivity measurements in tape punches (cpm) (upper) for dorsal skin after sulphur-35-cystine administration, are plotted against the time after injection of the labelled amino acids. The arrows mark the main points of reference for the transit experiments. Each point represents the mean and standard error of between 3 and 13 animals. The tape punch experiment used 153 mice while the skin punch experiment used 120 mice.

Fig. 4 shows the results obtained with the labelled amino acids. Forty minutes after exposure the label is distributed evenly over all nucleated (living) cells quite unlike the situation with ^{3}HTdR or ^{125}IUdR where the label is nuclear and is initially distributed only in the basal (proliferative) layers. Relatively little label is observed over the 'dead' cornified cells or over the dermal region. After 4–6 days, label can be observed scattered over the cornified cell layers (Figs 4B and D) i.e. the labelled 'front' has moved through several epidermal layers.

The time taken for the first labelled cells to move from the basal to the top granular layer is longer than the time taken to move through the same number of layers in the cornified regions in all regions studied, i.e. the transit *rate* measured by the nuclear label technique is longer than expected on the basis of the estimates of the transit rate through the differentiated layers. This is attributed to the post-mitotic maturation time in the basal layer, which can therefore be estimated from the data as shown in Table 2. The rate of movement once off the basal layer (i.e. as measured by the transit from granular layer to the surface) must be constant over a period of time and is the 'flow rate' through the differentiating compartment, and this can be equated to the cellular flow rate (the cellular input into

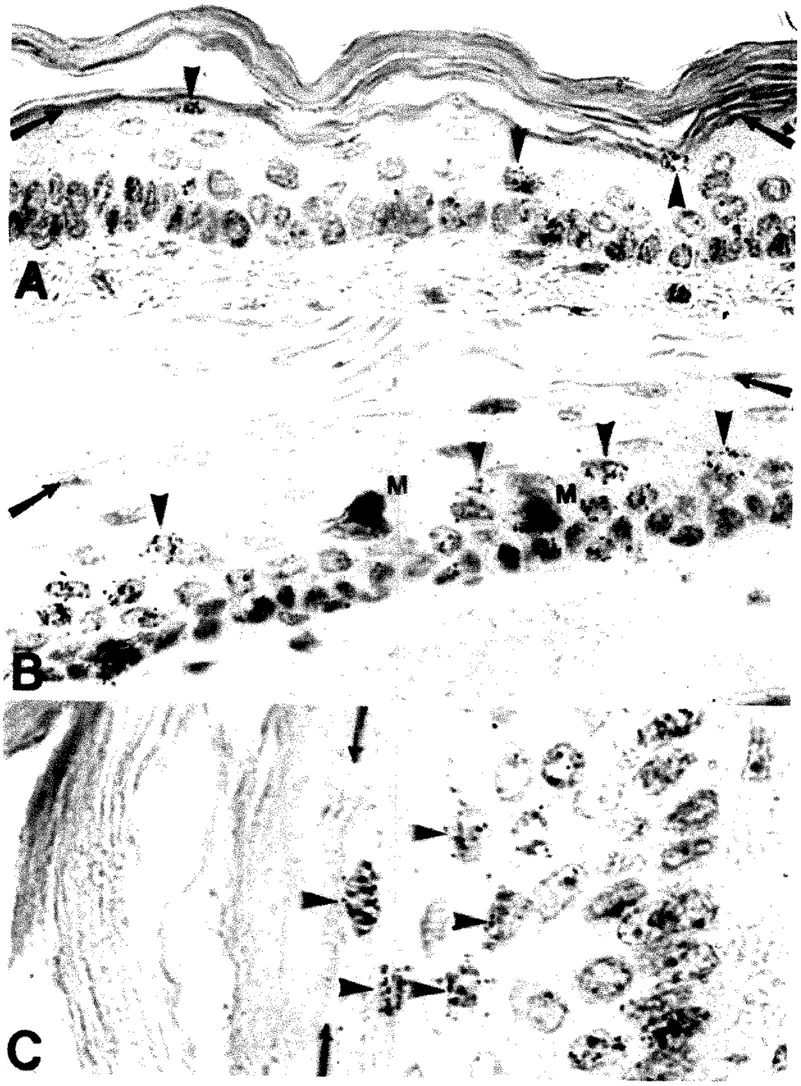

FIGURE 3. Autoradiographs of mouse epidermis prepared 4 days after injection of ^{3}HTdR showing suprabasal labelled cells (arrow heads) in ear (A), tail (B) and foot (C). In ear and foot, labelled cells can be found in the upper granular layer, i.e. at the boundary between the living differentiating cells and the dead cornified cells (arrowed). This represents movement through 3–4 layers in ear and 7–12 layers in foot. M, melanin.
(Magnifications; A ×680, B ×720, C ×800.)

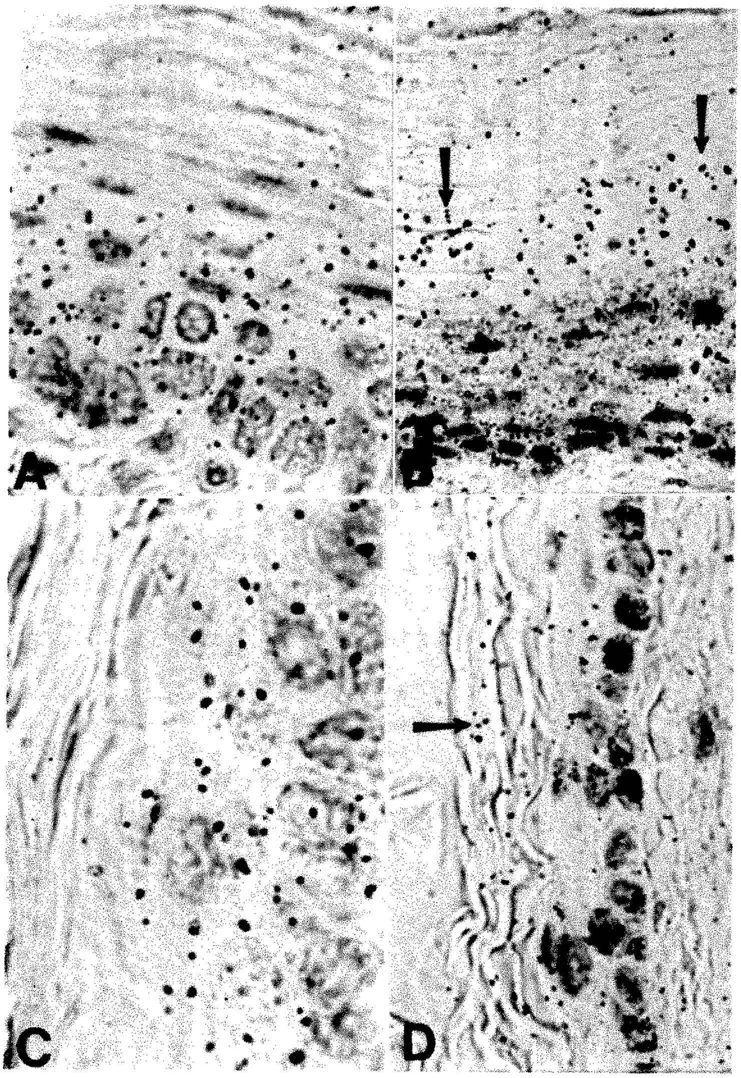

FIGURE 4. Autoradiographs prepared 40 min, 4 or 6 days after exposure to tritiated methionine. Forty minutes after injection in either foot (A) or ear (C) there are cytoplasmic grains over the basal and differentiating cell layers. Four days after exposure in the foot the 'front' of scattered grains has moved into the cornified layer (B) (arrowed). Six days after injection the ear sample (D) shows scattered grains in all layers of the epidermis.
(Magnifications; A ×9600, B ×880, C ×1040, D ×800.)

TABLE 2. Summary of epidermal transit measurements (days)

	Dorsal skin			Ear			Tail			Foot		
	Cell layers	Total	Transit days/layer	Cell layers	Total	Transit days/layer	Cell layers	Total	Transit days/layer	Cell layers	Total	Transit days/layer
(A)+(B) Basal to granular plus granular to surface	9	11	1·22	14·5	13·5	0·93	37·5	14	0·37	41	9·5	0·23
(C) Basal to surface	9	11	1·22	14·5	14·5	1·00	37·5	12	0·32	—	—	—
(D) Mean	9	11	1·22	14·5	14·0	0·97	37·5	13	0·35	41	9·5	0·23
(E) Transit rate once off basal layer			1·00			0·77			0·25			0·17
(F) Estimate of post-mitotic basal layer maturation time			2·0			3·0			3·8–4·0			2·0–2·5
(G) Estimate for true total transit			9			11			9			7
(H) Cell flow rate (cells/day)			1·0			1·3			4·0			5·9
(I) Estimated EPU basal population size			10·6			9·7			25			16–20
(J) Cell production rate (cells/h/100 basal cells)			0·39 (0·66)			0·56 (0·17)			0·67			1·38 (0·87)
(K) Orderedness of non-proliferative cells			Stacked			Stacked			Non-stacked			Non-stacked

(A), (B) and (C). From Table 1. A and C include post-mitotic basal layer maturation time (^{3}HTdR and ^{135}IUdR samples).
(E). Obtained from measurements of time taken to move from granular layer to surface (^{3}H-methionine and ^{35}S-cystine) (Table 1B—days/layer).
(F). Calculated from the difference in transit rate once off the basal layer and the transit rate measured when the post-mitotic maturation time is included, i.e. A and C. F = total transit time minus the product of transit rate once off the basal layer and the total number of layers.
(G). The total transit time corrected for the basal maturation time.
(H). Reciprocal of transit rate once in movement. This is equivalent to the cell production rate/day/EPU or the cell production rate/day/cornified cell.
(I). Calculated from figures in literature (Potten, 1974; Allen & Potten, 1975; Christophers *et al.*, 1974; Christophers & Laurence, 1973; and unpublished data) or measured on epidermal sheet preparations.
(J). Cell flow rate corrected for basal cell population size. The values in brackets are the average values that can be extracted from the literature (Potten, 1974; Christophers *et al.*, 1974; Christophers & Laurence, 1973; Blenkinsopp, 1968; Grube *et al.*, 1970; Tvermyr, 1969, 1972; Hegazy & Fowler, 1973; Sherman *et al.*, 1961; Laurence, 1973). $J = LI.\overline{T_s}N = MI.\overline{T_m}N$ where LI and MI are the labelling and mitotic indices, T_s and T_m are the lengths of S and M and N is the number of basal cells.

the differentiating layer). The cell flow rate can be expressed as cell production per hour per 100 basal cells and compared directly with cell production rates obtained from labelling or mitotic index data (Table 2).

DISCUSSION

These techniques have definite limitations and as yet statistical limits cannot be placed on the transit times determined; however, the results presented represent an attempt to quantify transit measurements more precisely and to extend the measurements to other regions of epidermis within animals of the same strain. The transit data can also be interpreted in relation to the epidermal proliferative units (EPU) (Potten, 1974; Allen & Potten, 1974). Each experiment, if considered in isolation from the rest, would not provide data of value, due partly to the large variability, but when compared with all the other experiments and considered with the autoradiographic studies the results can be more readily interpreted. Tape radioactivity measurements after ^{3}HTdR have previously been described as unsuitable for transit measurements (Iversen *et al.*, 1968) a conclusion the present data would support if the tape punch data were considered in isolation. The data in total provide a fairly reasonable picture of the *minimum* epidermal transit times. Estimates of the mean transit times cannot be made at present. The transit time is clearly related to the cell production rate and the total cell layers (thickness) and a study of all three parameters should give a consistent picture. Some indication of the degree of correlation based on the presently available data is given in Table 2.

The transit values are consistent with the tissues having *mean* cell flow rates differing by a factor of 6, as shown in Table 2. The values obtained for dorsal skin agree with the transit values obtained previously from hairless dorsum (basal to top granular layer) (Iversen *et al.*, 1968; Skjaeggestad, 1964; Downes, Matoltsy & Sweeney, 1967; Baden & Roth, 1965) and with the accumulated labelling or mitotic data (cell production rates) for various mouse strains (including hairless) and data for DBA-2 dorsum (unpublished observations; Potten, 1974; Christophers *et al.*, 1974; Christophers & Laurence, 1973; Blenkinsopp, 1968; Grube, Auerbach & Brues, 1970; Tvermyr, 1969, 1972; Hegazy & Fowler, 1973; Sherman, Quastler & Wimber, 1961). The transit measurement and cell production values for foot also agree with data obtained elsewhere (Christophers *et al.*, 1974, Christophers & Laurence, 1973).

There is a large difference in the ear data. Previous estimates for ear transit time provided values of about 20–24 days (Christophers *et al.*, 1974; Christophers & Laurence, 1973) while the present data would suggest transit times very similar to those for mouse dorsum (i.e. about 11 days). This is more difficult to explain on the basis of strain differences. Labelled cells definitely appear in some numbers in the upper granular layer 4 days after administration of ^{3}HTdR (Fig. 3A). Guinea-pig ear transit times are shorter and more comparable to those reported here (Christophers *et al.*, 1974). Ear epidermis labelling and mitotic indices are similar to those for dorsum and on this basis one would expect the transit times to be only slightly longer when account is taken of the slight differences in the number of cell layers in ear and dorsum (Table 1). The data would suggest that differences in strain, housing or handling procedures can cause rather large changes in basal maturation time or cell production rates and thus transit times. This is further suggested by earlier work using the same technique where total radioactivity measurements in a DBA-1 mouse suggested a basal to top granular cell transit time of 7 days rather than the 5 days observed in the present experiments (Potten, 1973). However this earlier estimate was based on an experiment not primarily designed to measure transit times and lacked sufficient experimental points.

ACKNOWLEDGMENTS

I am grateful to Irene Wylie, Margaret Grimes and Dorothy Robinson for their valuable technical assistance. The work was supported by grants from the Cancer Research Campaign and the Medical Research Council of Great Britain.

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Immunological responses of patients with psoriasis and the effect of treatment with methotrexate

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Accepted for publication 26 March 1975

SUMMARY

A group of thirty-six patients, of whom fourteen were being treated with methotrexate, were studied in order to assess T-lymphocyte function by *in vitro* techniques.

Circulating T-lymphocytes in aliquots of blood were assessed by the rosetting technique. No differences were found in psoriatics, whether on methotrexate or not, compared with fifteen control subjects.

Lymphocyte counts and lymphocyte transformation to phytohaemagglutinin (PHA) tended to be lower in the psoriatic group as a whole than in the controls, but the differences were not statistically significant. However, a significant inverse relationship was found between the extent of the skin lesions and lymphocyte transformation to PHA, i.e. the smaller the area of skin affected the higher the lymphocyte transformation. Psoriatics treated with methotrexate had fewer skin lesions and higher lymphocyte transformation to PHA than psoriatics not so treated, probably reflecting this inverse relationship. The reason why the presence of extensive psoriasis is associated with depressed lymphocyte transformation is not understood. No evidence was found that methotrexate depressed cell-mediated immunity as judged by these *in vitro* tests.

An immunological basis for the pathogenesis of psoriasis has been extensively investigated but no definite relationship found (Lorincz, 1958; Skog & Linder, 1965; Farber & McClintock, 1968). Serum proteins have been analysed in patients with psoriasis and the only consistent finding has been a decreased IgM level in one-third of the patients studied, especially in those with a concurrent arthritis (Fraser, Dick & Crichton, 1969). Where it has been measured, the antibody response of untreated patients appears normal (Epstein & Maibach, 1965). However, on low dose immunosuppressive therapy, the primary and secondary humoral antibody response to a new antigen (plague vaccine) was virtually abolished, whilst the secondary response to a previously encountered antigen was normal (Epstein, Maibach & Senecal, 1965).

In patients with psoriasis it is also difficult to induce delayed hypersensitivity to chemical agents such as dinitrochlorobenzene (DNCB). Epstein & Maibach (1965) noted that only 42% of psoriatics could be sentized with DNCB compared with 69% of control subjects. When the patients were receiving immunosuppressive agents such as azathioprine or methotrexate only 15% gave positive skin tests. In contrast, healthy control subjects given similar chemotherapy showed no decrease in their ability to be sensitized to DNCB. This raised the possibility that psoriatic patients in general were marginally immunodeficient in cell-mediated immunity and that treatment with methotrexate or azathioprine rendered them more obviously so.

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Based on this observation, the present study was undertaken to find out if there was any *in vitro* reflection of these clinical findings of deficient cell-mediated immunity, as tested by lymphocyte transformation with phytohaemagglutinin (PHA) and rosetting techniques (Brain, Gordon & Willetts, 1970) designed to show numbers of circulating T-lymphocytes (Roitt *et al.*, 1969).

PATIENTS, MATERIALS AND METHODS

Patients

Thirty-six patients with psoriasis were studied. The twenty-one not taking methotrexate, 6 males and 15 females, had an average age of 44 years. Fourteen were taking methotrexate in an average weekly dose of 21 mg, 7 males and 8 females, and had an average age of 51 years. One patient was taking azathioprine 75 mg daily. The extent of the skin lesions was graded arbitrarily as +, + + or + + +, where + represents 5–10% of the body area covered by lesions, to + + + where 75% or more of the skin was involved. Fifteen control subjects were obtained from the laboratory and medical personnel, 8 male and 7 female, average age 30 years.

Methods

Whole blood lymphocyte transformation

Fresh heparinized blood was diluted ten times in Eagle's MEM (Wellcome Reagents Ltd.) and phytohaemagglutinin (Wellcome Reagents Ltd.) at a final dilution of 1:150. Cultures of 0·2 ml of this suspension were set up in triplicate in microtitre trays, gassed with 5% CO_2 in air and cultured for 72 h at 37°C. Sixteen hours before harvesting, tritiated thymidine 0·1 μCi was added, specific activity 21·9 Ci/mmd (Radiochemical Centre, Amersham). The cells were then washed twice with cold phosphate buffered saline and transferred to filter paper discs (Whatman Ltd.). The discs were dried and placed in cold 10% trichloracetic acid for 20 min then washed successively with 3% perchloric acid, methanol and lastly ether. The discs were then placed in scintillation fluid for isotope counting. The results are expressed as a ratio of the counts per minute (cpm) per volume of blood culture compared with the same control subject studied on each occasion (whole blood transformation ratio). Also on each occasion, the lymphocytes were counted, and from this the cpm per million lymphocytes was calculated. These results were also expressed as a ratio, compared with the same control subject studied at the same time (ratio cpm per 10^6 lymphocytes).

Spontaneous sheep red cell rosettes

Defibrinated blood was incubated with carbonyl iron for 30 min at 37°C. The sample was then placed on a Ficoll-isopaque gradient and the mononuclear leukocytes isolated. These were washed three times in Eagle's MEM containing 10% fetal calf serum, incubated with fresh washed sheep red blood cells (ratio 1:20) for 5 minutes at 37°C, centrifuged at 125 *g* for 5 minutes and kept at 4°C overnight. The percentage of lymphocytes with 3 or more red cells attached—rosette forming cells—was then measured using acridine orange and ultraviolet microscopy (Brostoff, 1974).

RESULTS

Lymphocyte Studies

(a) *Whole blood transformation.* The ability of whole blood cultures to transform with mitogen depends on the number of lymphocytes contained in each sample. The lymphocyte count of the psoriatic patients, whether taking methotrexate or not, tended to be lower than the normals although as a group these differences were not significant (Fig. 1). This is reflected in the whole blood lymphocyte transformation ratio, where one-third of the psoriatic patients had a ratio less than the lowest normal control value (Fig. 2). There was no correlation between the extent of the skin involved and the whole blood transformation ratio.

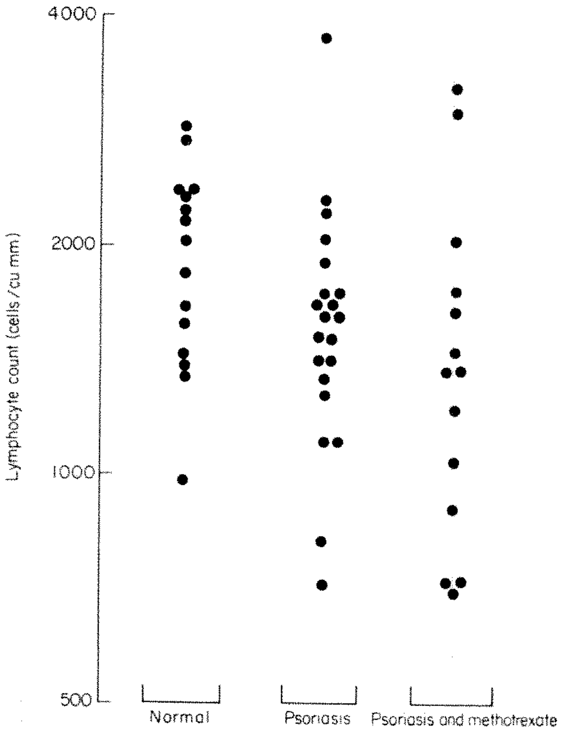

FIGURE 1. Lymphocyte count in patients with psoriasis compared with normal controls. The patients tend to have a lower lymphocyte count than the controls, whether or not they are taking methotrexate.

(b) *Lymphocyte transformation.* If the thymidine incorporation is related to the actual number of lymphocytes in each sample, a figure is obtained that reflects the ability of the lymphocyte population to respond and not only the aliquot of 'whole blood'. The results of the ratio of ^{3}H thymidine incorporation of the patients' lymphocytes compared with the same control subjects on each occasion are shown in Fig. 3.

The ratio of thymidine incorporation of either group of patients is not significantly different from the controls ($P = 0{\cdot}1$). However, patients not taking methotrexate tend to have a lower ratio than the controls, and those taking methotrexate a higher one. There is a significant difference between the patient groups, those on methotrexate having a significantly higher ratio than those not on this treatment ($P = 0{\cdot}025$).

(c) *Correlation of lymphocyte transformation with the extent of the skin lesions.* The observation that patients treated with methotrexate showed greater lymphocyte transformability than those not taking the drug was surprising. However, as a result of this therapy, the treated patients had less skin involvement than the others (Fig. 4). If the lymphocyte transformation is correlated with the extent of the skin lesions, irrespective of treatment, it can be seen that the greater the area of skin affected, the lower the transformation ratio, untreated patients tending to have the more extensive lesions. All but two of the patients not on methotrexate had a transformation ratio less than 1.

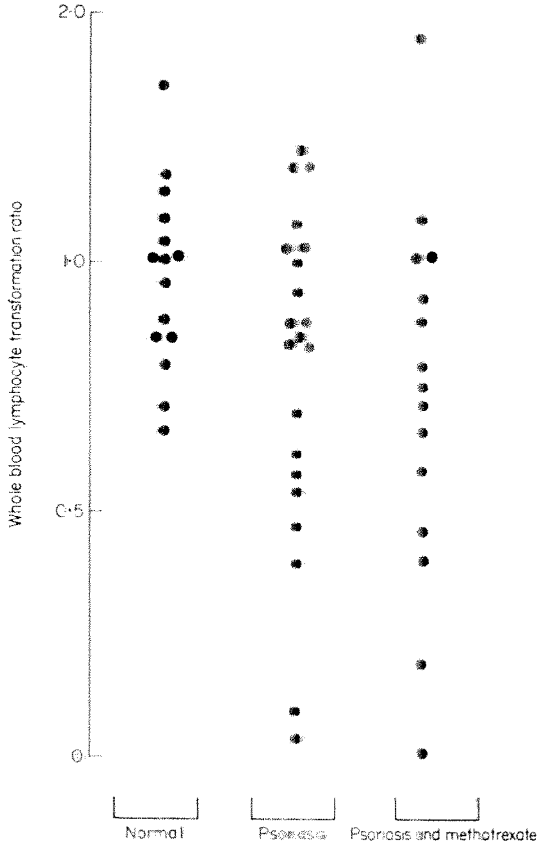

FIGURE 2. Whole blood transformation ratio in psoriatic patients and controls. One-third of the patients have a ratio less than the lowest normal control. These figures reflect the lower lymphocyte count found in these patients.

(d) *Rosette forming cells.* In view of the clinical finding that patients with psoriasis on immunosuppressives were difficult to sensitize with DNCB it was of interest to measure the T-lymphocytes in these patients compared with controls not taking this drug. There was no significant difference in the percentage of sheep red cell binding lymphocytes in the circulation of patients compared with controls (Fig. 5).

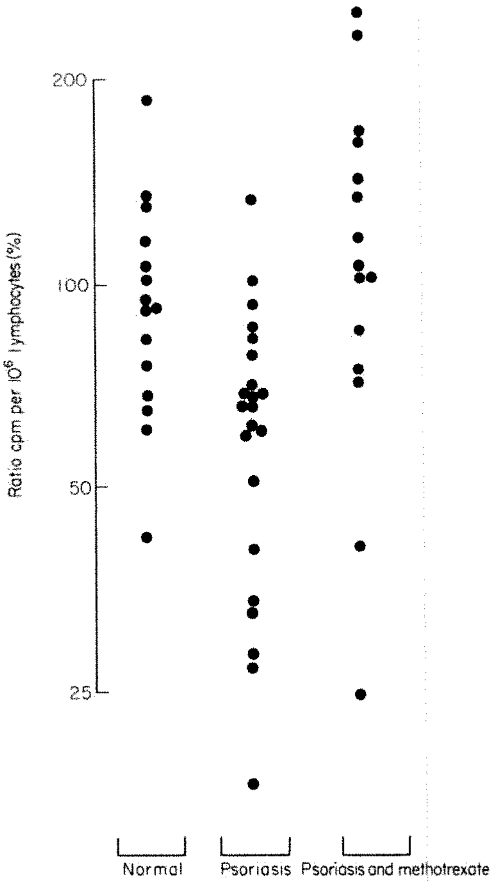

FIGURE 3. Lymphocyte transformation of either group of patients is not significantly different from controls, but patients on methotrexate give a significantly higher transformation ratio than those not taking this treatment, $P = 0{\cdot}025$.

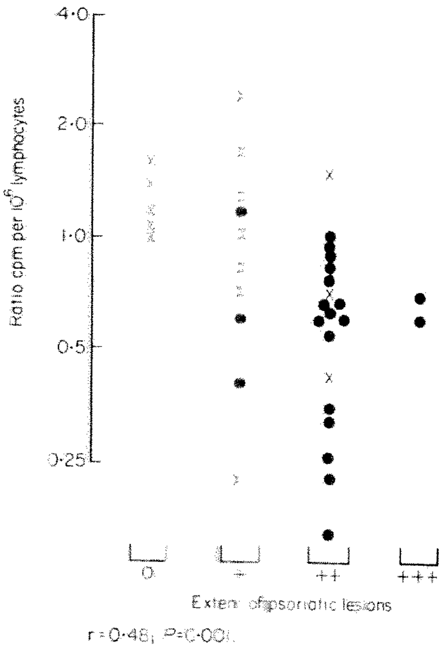

FIGURE 4. Correlation between extent of skin lesions and lymphocyte transformation with PHA. The greater the area of skin affected by psoriasis, the lower the transformation ratio. Patients taking methotrexate (x) tended to have less skin involved and a higher transformation ratio than those not on this treatment (●).

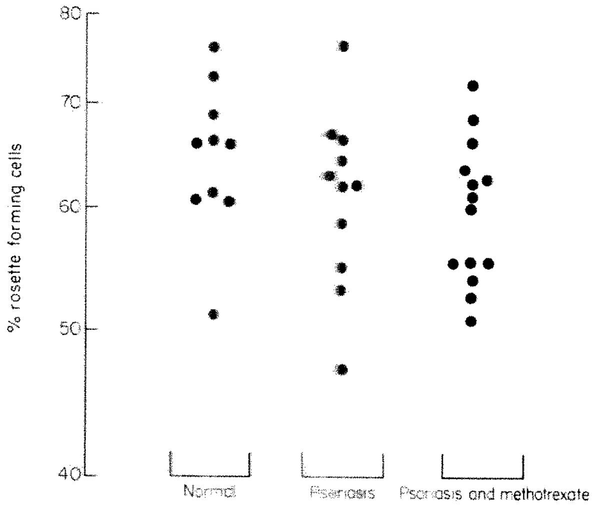

FIGURE 5. There is no significant difference in the T-lymphocytes, as measured by sheep red cell rosettes, between the patients and control subjects

DISCUSSION

Using *in vitro* tests to measure the number of circulating T-lymphocytes, we have been unable to find any gross difference between psoriatic patients and control subjects. However, the whole blood transformation ratios of the psoriatic patients, whether or not they were taking methotrexate, were lower than the normal control ratios. This was probably due to the smaller number of circulating lymphocytes in these patients. When the actual ability of each lymphocyte to transform is calculated, the picture is different. The psoriatic patients as a whole do not differ from the normal controls, but there is a significant difference between the patient groups. Patients on methotrexate have higher lymphocyte transformation ratios than untreated patients. This is a surprising result, but we have also found that the more extensive the skin lesions, the lower is the lymphocyte transformation ratio. If patients are further subdivided, it becomes clear that the patients being treated with methotrexate have less skin involved as a result of treatment although their original condition was more severe to warrant such treatment, thus explaining these findings (Fig. 4). The explanation for the diminished lymphocyte response in patients with extensive skin lesions is unclear. A number of undefined factors do influence the ability of peripheral blood lymphocytes to respond to mitogen. Recent surgical procedures reduce the transformation of patients' lymphocytes to PHA (Riddle & Berenbaum, 1967). Similarly, repeated anaesthesia without surgical intervention also impairs this response in both animals and man (Viljanen et al., 1973; Espanol, Todd & Soothill, 1974). Thus many non-specific factors may play a part in altering the mitogenic response, those being involved in psoriatic patients still remaining undefined.

The quantitative differences in lymphocyte reactivity that we have found in patients with psoriasis seem specifically related to skin involvement. Other authors have found that psoriatics when treated with immunosuppressives do not seem to be able to respond to new antigens, whether by antibody production or delayed hypersensitivity, although their ability to mount a secondary response is normal. Our studies with lymphocyte transformation and rosette tests have not identified the reasons for the abnormally low response to new antigens in psoriatics found by other authors. It would be interesting if, in the previous studies, the non-responders were particularly those patients with extensive skin involvement. The observations of diminished immunological reactivity in these patients are fascinating and require both confirmation and further investigation.

ACKNOWLEDGMENTS

We should like to thank Mrs Sue Pack for her excellent technical assistance, and Dr G.M.Levene for allowing us access to patients under his care. JB is supported by the Medical Research Council.

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British Journal of Dermatology (1975) **93**, 667.

Studies on the energy metabolism in lichen planus

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Accepted for publication 24 March 1975

SUMMARY

Various epidermal enzymes and cofactors were measured in patients with lichen planus and in healthy controls with the aid of Lowry's microtechniques, including enzymatic cycling. The steady-state levels of the nicotinamide adenine dinucleotides NAD* and NADP were decreased and this was evident even in areas still free from lesions. The oxidized and reduced portions of NAD were altered indicating changed equilibria of NAD dependent dehydrogenases. Reduced NADP was more tightly controlled at the normal level which is regarded as evidence of an unaltered biosynthetic potential in this disease. In conjunction with earlier data the results indicate a preserved glycolytic and pentose shunt activity while the mitochondria display signs of dysfunction.

Enzymatic changes in psoriasis, lichen simplex, lichen planus and pityriasis rosea have been reported recently (Hammar, 1973c). Lichen planus was found to differ in many respects from psoriasis and lichen simplex especially in relation to mitochondrial functions. Some further enzymes and cofactors were therefore measured in order to cover different aspects of epidermal energy metabolism in lichen planus.

MATERIALS AND METHODS

Punch biopsies were collected without anaesthesia from the forearm in fourteen patients with lichen planus and in eighteen healthy controls. The mean age of the patients was 43 years, and of the con-

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* *Abbreviations:*
ADP, adenosine diphosphate; AK, adenylate kinase (EC 2. 7. 4. 3); ATP, adenosine triphosphate; FH, fumarate hydratase (EC 4. 2. 1. 2.); GAPDH, glyceraldehyde phosphate dehydrogenase (EC 1. 2. 1. 12); G3PDH, glycerol-3-phosphate dehydrogenase (EC 1. 1. 1. 8.); G6PDH, glucose-6-phosphate dehydrogenase (EC 1. 1. 1. 49); HOADH, 3-hydroxyacyl-CoA dehydrogenase (EC 1. 1. 1. 35); ICDH(NAD) NAD dependent isocitrate dehydrogenase (EC 1. 1. 1. 41); ICDH(NADP) NADP dependent isocitrate dehydrogenase (EC 1. 1. 1. 42); LDH, lactate dehydrogenase (EC 1. 1. 1. 27); MDH, malate dehydrogenase (EC 1. 1. 1. 37); NAD, nicotinamide adenine dinucleotide, NAD^+ oxidized, NADH reduced form; NADP, nicotinamide adenine dinucleotide phosphate, $NADP^+$ oxidized, NADPH reduced form.

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trols 37 years. The patients had had their disease for 1–12 months prior to biopsy. The mean was 4 months. None of them was treated during the month immediately preceding biopsy. Apart from the skin disease the patients considered themselves healthy. The controls included three subjects who had been treated a short time previously for a localized dermatitis; the others were medical students or hospital personnel. At the time of biopsy they declared themselves healthy and gave a negative history of psoriasis, lichen simplex and lichen planus as regards both themselves and their families.

In all cases the biopsies were taken from the extensor region of the forearm; in the patients with lichen planus one was taken from the centre of a lesion and another from the non-involved skin 40 mm away from the lesion. The further preparation of the material, including dissection and weighing, was done according to Lowry & Passonneau (1972) as described earlier (Hammar, 1970, 1973 c). The dissected specimens contained the subcorneal and the basal part of the same rete ridges. This material was processed in 4–7 samples to counteract variation. In the non-involved skin epidermal material was mostly divided into two parts representing the subcorneal and basal layers, each with a height of about 3–5 cells. If the epidermis was thicker, the central portion was omitted, thus leaving the height of the specimens unchanged. Care was taken to avoid contamination with connective tissue papillae adjacent to the epidermis.

Enzyme assays. The assays were carried out by the principles of Lowry & Passonneau (1972). Details of the assays of G3PDH are given by Hammar (1973d), and of LDH and MDH by Alvares *et al.* (1972).

Cofactor assays. For the quantitative measurements of NAD and NADP, enzymatic cycling according to Lowry *et al.* (1961) was used. 0·4–0·6 μg of dissected material was introduced into 0·76 μl cold 0·02 mol/l NaOH containing 1 mmol/l cysteine. Sometimes it was necessary to reverse this sequence. For extraction of the dinucleotides either way could be used. The latter procedure gave the same result as the former when the total dinucleotide content was quantified. In the reversed procedure, however, the reduced dinucleotides were oxidized substantially. Total NAD and total NADP were measured in the alkaline suspension without further treatment. For assaying the reduced dinucleotides, the suspension was first heated for 20 min at 60°C. 7·96 μl of the appropriate cycling reagent was then added. The cycling time was 90 min at 38°C, and cycling was stopped by heating for 3 min at 100°C. This was followed by incubation for 15 min at 25°C after the addition of 7·96 μl of the appropriate developer reagent. The reaction was stopped by adding 1·99 μl 5 mol/l HCl. A 5 μl aliquot was added to 25 μl 7 mol/l NaOH, and the mixture was heated for 10 min at 60°C and diluted with 200 μl redistilled water. The fluorescence obtained was read on a Farrand Ratio Fluorometer. Appropriate standards and blanks were taken throughout the procedure. Reduced dinucleotides, in at least two concentrations, were used to check the linearity of the reaction. Checks were also made to secure a magnification of the cycling reaction in the order of about 4000 times for NAD and 15000 times for NADP. In this way most readings of actual tissue contents were more than twice those of the blanks.

Chemicals. Analytical reagents were obtained from Boehringer, Mannheim, GmbH, BRD, and Sigma Chemical Co., St Louis, USA. The enzymes used in the cycling reagents were purified when necessary and adjusted to a proportion which gave the desired yield, as followed by the recommendations of Lowry *et al.* (1961).

Statistical procedures. Significances of differences were obtained by t-tests or by analysis of variance according to standard procedures (Snedecor, 1957). Multiple comparisons with one control were made as described by Dunnett (1964). Correction for unequal group size was made according to Seeger (1966). The experimental error was measured on 4–7 samples taken from the same biopsy sections and expressed as coefficient of variation. This was 20% for G3PDH, 25% for LDH, 29% for MDH, 21% for total NAD, 26% for NADH and 15% for both total NADP and NADPH.

RESULTS

Enzymes. The mean activities of G3PDH, LDH and MDH are summarized in Table 1.

The activity of G3PDH was significantly lower in the involved epidermis, as compared with non-involved skin ($P<0\cdot01$). In both involved and non-involved epidermis the activity was lower in the subcorneal than in the basal layers ($P<0\cdot02$). The levels of enzymatic activity were decreased in both non-involved and involved skin of the patients with lichen planus, in relation to earlier findings in control subjects (Hammar, Thyresson & Brolin, 1968).

TABLE 1. Mean activities of glycerol-3-phosphate, lactate and malate dehydrogenases in epidermis expressed as mol of substrate consumed per kg dry weight per h of incubation at 38° C. Standard errors of the mean are indicated

				Lichen planus	
Group	No.	Controls	No.	Non-involved	Involved
G3PDH					
Subcorneal		—	7	0·058 ± 0·005	0·040 ± 0·005
Basal	7	0·123 ± 0·013*	7	0·084 ± 0·004	0·051 ± 0·004
LDH					
Subcorneal	10	29·9 ± 4·3	7	34·2 ± 2·3	38·3 ± 5·0
Basal	10	36·8 ± 4·1	7	29·3 ± 1·7	52·2 ± 4·2
MDH					
Subcorneal	6	24·5 ± 3·6	7	30·2 ± 2·8	17·0 ± 2·0
Basal	6	25·3 ± 3·0	7	29·3 ± 2·5	27·1 ± 3·7

* Taken from Hammar, Thyresson & Brolin (1968).

The activity of LDH was similar in the non-involved epidermis to that in controls; no difference was found between the two layers studied. In the involved skin the activity of LDH was increased in the basal layers. The difference in activity between the lesion and the controls and between the lesion and the non-involved skin was significant ($P<0\cdot01$).

The activity of MDH in the non-involved epidermis was similar to that in the controls. In the involved epidermis the activity in the subcorneal layers was lower than that in the basal layers, and was also lower than the levels in the two groups of unaffected skin ($P<0\cdot02$).

Nicotinamide adenine dinucleotides. Marked changes in the total contents of NAD and NADP were found in the involved epidermis, as shown in Table 2. The total dinucleotide levels in the lesion were significantly decreased when compared both with the non-involved and with the control epidermis ($P<0\cdot001$). Variation was encountered, however, when the oxidized and reduced components of the dinucleotides were examined separately.

NAD. A decrease in NADH in the non-involved epidermis was evident, as shown in Table 2, and still lower levels were also found in the involved skin. The decrease in total NAD was mostly due to NADH. It may be noted that the proportion of the total NAD comprising NADH decreased significantly from about 40% in the controls to 30% in both non-involved and involved skin of the lichen planus patients ($P < 0{\cdot}005$). No differences were observed between the two layers examined.

NADP. In the subcorneal layers the oxidized component was decreased to some extent in the non-involved epidermis, and more markedly in the lesion, when compared with the controls (Table 2).

TABLE 2. Nicotinamide adenine dinucleotides in epidermis. The contents are expressed as mmol per kg dry weight. Means and their standard errors are given. The products were calculated on data from the same individual. Since only a few patients with lichen planus were included, calculation of products was also performed from the means (a)

Group	No.	Controls	No.	Lichen planus	
				Non-involved	Involved
NAD⁺					
Subcorneal	13	0·66 ± 0·05	8	0·83 ± 0·08	0·56 ± 0·08
Basal	13	0·68 ± 0·06	8	0·78 ± 0·11	0·62 ± 0·05
NADH					
Subcorneal	13	0·46 ± 0·04	8	0·34 ± 0·04	0·22 ± 0·03
Basal	13	0·51 ± 0·05	8	0·35 ± 0·05	0·28 ± 0·04
Quotient: (NAD⁺)/(NADH)					
Subcorneal	13	1·69 ± 0·26	8	2·77 ± 0·46	3·02 ± 0·67
Basal	13	1·63 ± 0·29	8	2·69 ± 0·63	2·61 ± 0·51
NADP⁺					
Subcorneal	12	0·030 ± 0·008	8	0·017 ± 0·005	0·012 ± 0·005
Basal	12	0·035 ± 0·007	8	0·040 ± 0·008	0·016 ± 0·006
NADPH					
Subcorneal	12	0·123 ± 0·008	8	0·118 ± 0·006	0·096 ± 0·006
Basal	12	0·104 ± 0·005	8	0·101 ± 0·009	0·104 ± 0·004
Quotient: (NADP⁺)/(NADPH)					
Subcorneal	12	0·29 ± 0·09	8	0·16 ± 0·06	0·13 ± 0·06
Basal	12	0·37 ± 0·08	8	0·43 ± 0·11	0·16 ± 0·06
Product of quotients: (NADH)·(NADP⁺)/(NAD⁺)·(NADPH)					
Subcorneal	12	0·25 ± 0·09	3	0·03 ± 0·02	0·04 ± 0·04
	(a)	0·24 ± 0·12	(a)	0·06 ± 0·06	0·04 ± 0·04
Basal	12	0·36 ± 0·14	3	0·16 ± 0·08	0·06 ± 0·03
	(a)	0·33 ± 0·15	(a)	0·16 ± 0·10	0·06 ± 0·04

The levels of NADP⁺ in the lichen planus patients were probably different from the controls ($P < 0{\cdot}05$). The content of NADPH in the subcorneal layers was diminished only in the lesion ($P < 0{\cdot}01$).

In the basal layers a decrease of NADP⁺ was noted only in the lesion, while the NADPH levels were not altered (Table 2).

The relative content of NADPH expressed as percent of total NADP increased from about 80% in the controls to above 90% in the lichen planus lesion. The non-involved epidermis in the patients did not differ from that of the controls in this respect. The increase in the proportion of NADPH in the lesion differed significantly from that in the two groups of unaffected skin ($P < 0{\cdot}001$).

Redox ratios. The quotients between oxidized and reduced dinucleotides are presented in Table 2. For NAD the quotients were higher in lichen planus than in the controls, whereas for NADP the reverse was found. The product of the quotients (NADH)/(NAD$^+$) and (NADP$^+$)/(NADPH) obtained from the same individual showed a decrease in three patients with lichen planus when compared with twelve controls. The lowest values were found in the lesion. The difference between the controls and the two lichen planus groups was significant ($P<0{\cdot}01$). The products obtained by multiplying the means of the ratios (NADH)/(NAD$^+$) and (NADP$^+$)/(NADPH) were similar to those obtained in the three patients. Both products are given in Table 2.

DISCUSSION

The present results are summarized, together with those of earlier studies on lichen planus, in Table 3

TABLE 3. Metabolic changes in the epidermis in patients with lichen planus. Non-involved and involved skin of the patients with lichen planus was compared with controls, and involved and non-involved skin of the patients were compared with each other. The results were expressed as ratios. Ratios are only indicated in the table for those comparisons in which a significant difference was obtained between the members of the quotient. Non-significant differences are indicated by dashes.

The number 2·2 for GAPDH, for example, means an increased activity by 2·2 fold in the subcorneal layer of the lichen planus lesion over the control level. A number less than unity means decreased activity or content

Metabolic component	Comparison with healthy controls				Comparison with non-involved skin		Ref. of the author
	Non-involved		Involved				
	Subcorneal	Basal	Subcorneal	Basal	Subcorneal	Basal	
Glycolysis							
GAPDH	—	—	2·2	1·9	1·9	1·6	(1973 b)
LDH	—	—	—	1·4	—	1·8	present paper
G3PDH	—	0·7	—	0·4	0·7	0·6	
Pentose shunt							
G6PDH	—	—	—	1·8	1·4	2·0	(1971)
Citric acid cycle							
ICDH(NAD)	0·7	—	—	1·2	—	1·9	(1973 c)
ICDH(NADP)	—	—	0·5	—	0·5	—	(1973 c)
FH	—	—	—	—	—	1·4	(1973 c)
MDH	—	—	0·7	—	0·6	—	present paper
Lipolysis							
HOADH	—	—	0·4	0·5	0·3	0·5	(1971)
Interconversion of cofactors							
AK	—	—	1·3	1·2	1·5	1·3	(1973 b)
Cofactors							
ATP	—	—	—	—	—	—	(1973 a)
ADP	—	—	—	—	—	—	(1973 a)
NAD	—	—	0·7	0·7	0·7	0·8	present paper
% NADH	0·7	0·7	0·7	0·7	—	—	
NADP	—	—	0·7	0·8	0·8	0·8	
% NADPH	—	—	1·3	1·3	—	1·3	

(Hammar, 1971, 1973a,b,c). In the Table significant differences are given as ratios between activities or levels in the patients with lichen planus and those in healthy controls. The ratios between involved and non-involved epidermis are also included.

The glycolytic enzymes GAPDH and LDH showed increased activities in involved skin, as also did the pentose shunt enzyme G6PDH. This applied especially to the basal layers. In the lesion the citric acid cycle enzymes ICDH(NAD) and FH displayed slight increases in activity, while the opposite was noted for ICDH(NADP) and MDH.

The mitochondrial enzyme HOADH and G3PDH, an enzyme linking glycolysis to lipid metabolism, displayed decreased activities.

The cofactor ATP was not changed and only minor alterations of the ATP content were found during anoxia, even when prolonged to 15 and 30 min (Hammar, 1973a). The steady-state levels of total NAD and NADP were decreased and this included also NADH (Tables 2 and 3).

To judge by the ATP levels in the epidermis of the lichen planus lesion it seems that the glycolytic capacity is sufficient to maintain a steady-state level of ATP near that of the controls. On the other hand, the results indicate disturbances in mitochondrial function. These were also evident in an electron microscopical study by Johnson & Fry (1967) who showed during the natural course of lichen planus that mitochondria decreased in number and that their cristae diminished or disappeared. The altered redox quotients of the measured dinucleotides in the lichen planus lesion indicate other equilibria of various cytosolic and mitochondrial dehydrogenases than those found normally.

Minor variation was encountered in the NADPH contents. Generally, NADPH is used in the cell for biosynthesis (Horecker, 1967). Thus, the epidermal cells in lichen planus seem to modify the metabolism of this dinucleotide in order to maintain sufficient NADPH for their necessary biosynthetic operations.

The findings may be compared with observations during wound repair. The condition in lichen planus is in some respects similar to this situation, since it has been suggested that the damaged basal layer within areas of liquefaction degeneration in lichen planus is reconstituted by an active cell migration from the fringes of the lesion (Marks, Black & Wilson Jones, 1973). In the repair of an incisional wound the migrating epidermal cells show and increase both in biosynthetic and in mitotic activity. As summarized by Im & Hoopes (1970), increased glycolysis as well as enhanced activity of the pentose shunt are evident in wound repair. These authors found that the rate of formation of ATP and of reduced nicotinamide adenine dinucleotides was doubled and also that the steady-state levels of these nucleotides were increased. The glucose utilization occurred predominantly through glycolysis. The mitochondrial activity in this respect was decreased.

Our results differ in several respects from the findings in wound repair, indicating more complex biochemical events in the lichen planus lesion. Significant alterations were also found in the non-involved skin of the patients with lichen planus, as shown in Table 2. The product (NADH) $(NADP^+)$/ (NAD^+)(NADPH), which expresses the equilibria of various transhydrogenases (EC 1.6.1.1.), demonstrates this point. The biochemical changes found in lichen planus were also quite different from those observed in psoriasis and lichen simplex (Hammar, 1973c, 1975a,b). Such differences might be utilized in further investigations of the biochemical basis of these diseases.

ACKNOWLEDGMENTS

This investigation was supported by the Swedish Medical Research Council (19X-2593), by the Foundation of Edvard Welander and of the Karolinska Institute and by the Swedish Psoriasis Association, Stockholm.

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British Journal of Dermatology (1975) **92**, 675.

The lichen planus-like eruption after bone marrow transplantation

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Accepted for publication 1 April 1975

SUMMARY

A lichen planus-like eruption was seen in four patients after bone marrow transplantation. The skin and mucous membrane appearance closely mimicked lichen planus. The histopathology was also very similar to lichen planus. The occurrence of a lichen planus-like eruption (LPLE) after an immune basal cell damage related to the graft-versus-host reaction raised the question of the immune nature of this eruption. The correlation found between biological signs of graft-versus-host reaction and the outbreak or relapse of the lichen planus-like eruption supports the hypothesis that the skin changes could be a sign of a chronic immune response against recipient epidermis.

Despite compatibility to the major histocompatibility complex, a graft-versus-host reaction (GVHR) is often observed after allogenic bone marrow transplantation in man. GVHR is an immunological reaction between donor immuno-competent lymphoid cells and host tissues. The exact mechanism of the reaction and the antigen responsible for it are so far partially unknown (Slavin & Santos, 1973; Thomas, 1974). The main damage occurs in the skin, gastrointestinal tract and liver. In the skin, the target seems to be the basal cell layer (Lerner *et al.*, 1974). Clinically this damage is manifested by a maculo-papular or a dry scaling scarlatiniform rash or even an epidermal necrolysis (Peck, Herzig & Elias, 1972). These skin lesions are associated with the early phase of GVHR; but their evolution has been poorly documented because, in most of the cases, death has occurred rapidly. Recently, treatment with anti-thymocyte globulin has improved the survival of such patients and given the opportunity to observe chronic forms of GVHR (Storb *et al.*, 1974).

We have previously briefly reported the occurrence of a lichen planus-like eruption (LPLE) in one patient with a chronic form of GVHR (Saurat *et al.*, 1975). Since then we have discovered three additional cases and this report describes the clinical and histological features of the LPLE in four bone marrow transplanted patients. Correlations between the occurrence of LPLE and the immunological status of these patients were made.

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CASE REPORTS

Case I was briefly reported previously (Saurat *et al.*, 1975). This 13-year-old boy was admitted with post-hepatitis aplastic anaemia. Transplantation was performed with the marrow of his sister (HLA identical and non-reactive in mixed leukocyte culture). Extra dermatological data are reported in detail elsewhere (Gluckman *et al.*, 1975). Skin involvement showed two stages:

(I) On day 11 after transplantation, coincident with evidence of marrow engraftment, a maculo-papular, pruritic and erythematous rash appeared on the face and the neck and then spread to the whole body. A skin biopsy on day 11 was interpreted as a grade II GVHR (Lerner *et al.*, 1974); it showed vacuolar degeneration of basal cells and premature individual cell keratinization; cellular infiltration in the upper dermis was very sparse. This rash persisted to day 50 but scaling and hyper-pigmentation were obvious on day 25.

(II) On day 40 the lichenoid pattern began. Initially the lichenoid aspect was noted in the inner surface of the cheeks resulting in an *en reseau* type according to Laufer & Kuffer (1970). Skin lesions on his arms and feet were shiny, violaceous but with reticular arrangement and were slightly papular. Histology of this lesion on day 56 showed irregular hypergranulosis, slight acanthosis and a sparse infiltrate in the dermis; vacuolar degeneration of basal cells was present and was interpreted as a persisting grade II GVHR (Lerner *et al.*, 1974). Later as the buccal lichenoid lesions became ulcerative and painful, the whole skin surface was involved, including the palms and soles (Plate 1a), resulting in a lichenoid erythroderma. Papules were more obvious, becoming confluent in a reticular pattern on a deeply pigmented background (Plate 1b). Nails were severely damaged. Pruritus was present.

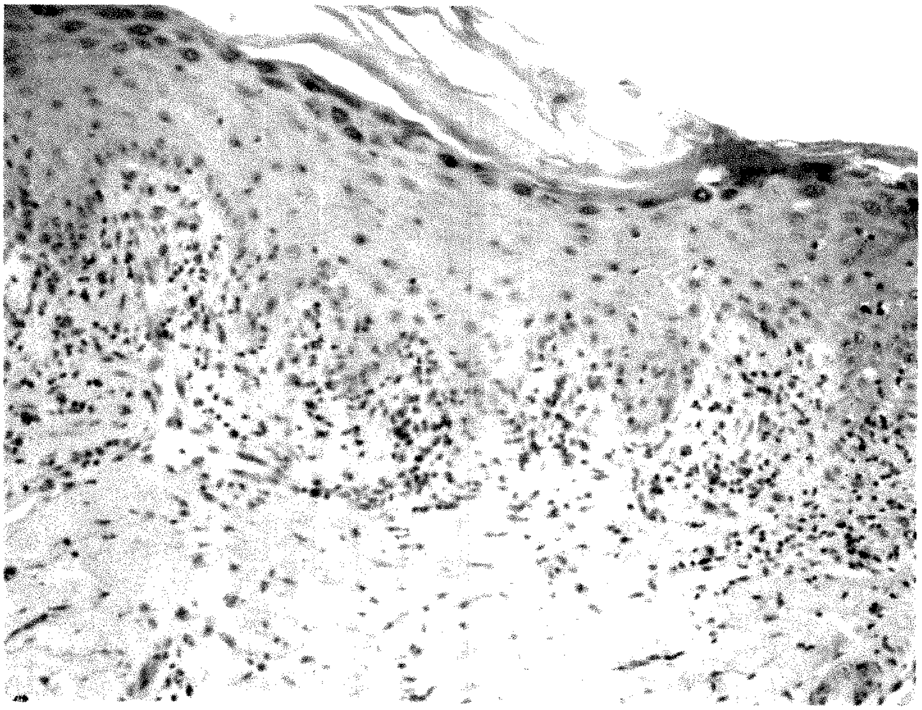

FIGURE 1. Histology of a papule on the abdomen on day 90 (H&E × 100).

Histology of a papule on day 90 showed orthokeratosis, irregular increase in thickness of the granular layer, acanthosis, colloid bodies and cell necrosis in the basal cell layer and a mononuclear cell infiltration immediately below the epidermis with pigmentary deposits (Fig. 1).

This severe lichenoid erythroderma became the main clinical problem, mouth, palm and sole involvement causing great discomfort. Prednisone (2 mg/kg/24 h) was given on day 105. The lichenoid eruption faded and the mouth erosions healed. On day 200, the lichenoid papules had disappeared, the mucous membranes were normal and the nails were regrowing normally. Diffuse hyperpigmentation with patches of depigmentation remained. Histology showed skin atrophy, persistent basal cell necrosis and dermal pigmentary disturbances as in the 'quiescent aggressor lymphocytes destructive lesion' described by Slavin & Santos (1973). On day 210, when the prednisone dose was 0·5 mg/kg/24 h, a few lichenoid papules with typical Wickham's striae appeared again on the dorsa of both hands and on the ankles. Mouth lesions relapsed with ulceration. One mg/kg/24 h of prednisone again improved the lesions.

Case II. This 30-year-old man was admitted for acute myeloblastic leukaemia. Transplantation was performed with the marrow of his matched brother (HLA identical and non-reactive in mixed leukocyte culture). As in case I, skin symptoms after the transplantation occurred in two stages.

(I) On day 24 a pruriginous maculo-papular rash appeared on the face, neck and thighs. Skin biopsy showed basal cell layer damage of a grade II GVHR (Lerner *et al.*, 1974). Marked hyperpigmentation superseded the erythematous lesions.

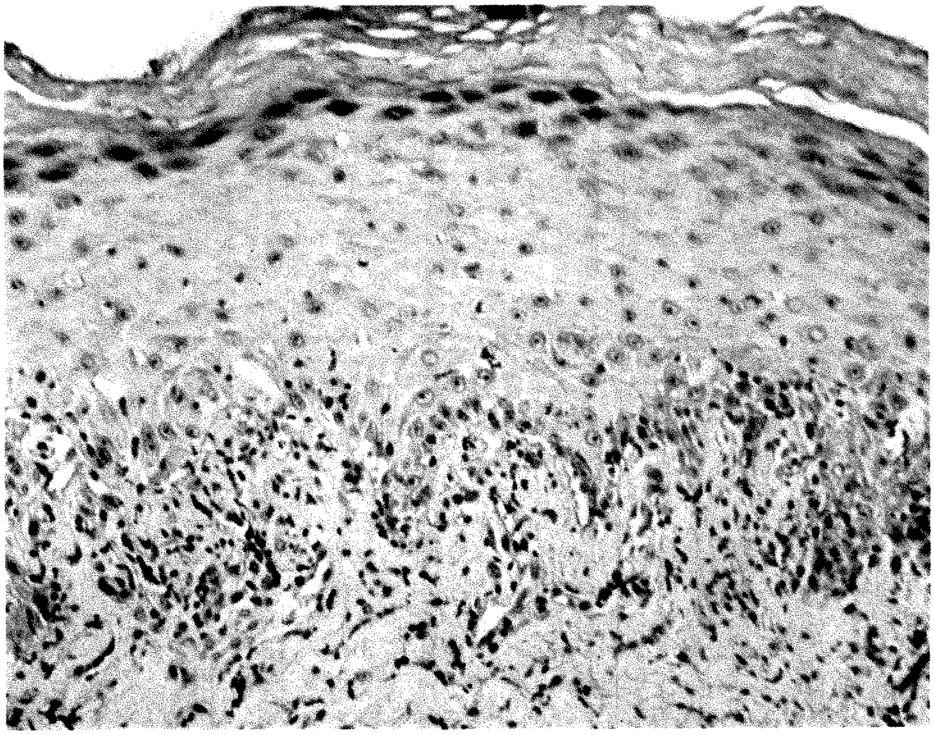

FIGURE 2. Histology of the papule shown in plate 1d. Case II 46 days after the graft (H&E × 100).

(II) On day 33 lichenoid *en réseau* lesions appeared on the inner surface of the cheeks (Plate 1c). The palate was typically involved. On day 45 a few typical lichenoid papules, slightly pruriginous, with Wickham's striae were seen on the dorsa of both hands (Plate 1d) and on the soles. Skin biopsy on day 46 showed orthokeratosis, irregular hypergranulosis and slight acanthosis. The dermal–epidermal junction showed important changes—basal cell necrosis, colloid bodies, and numerous clefts. The mononuclear infiltrate was not very intense but showed a sharp lower margin (Figs 2 and 3). Prednisone was given (1 mg/kg/24 h) and the lichenoid eruption gradually improved. On day 180 only the inner aspect of his cheeks remained lichenoid.

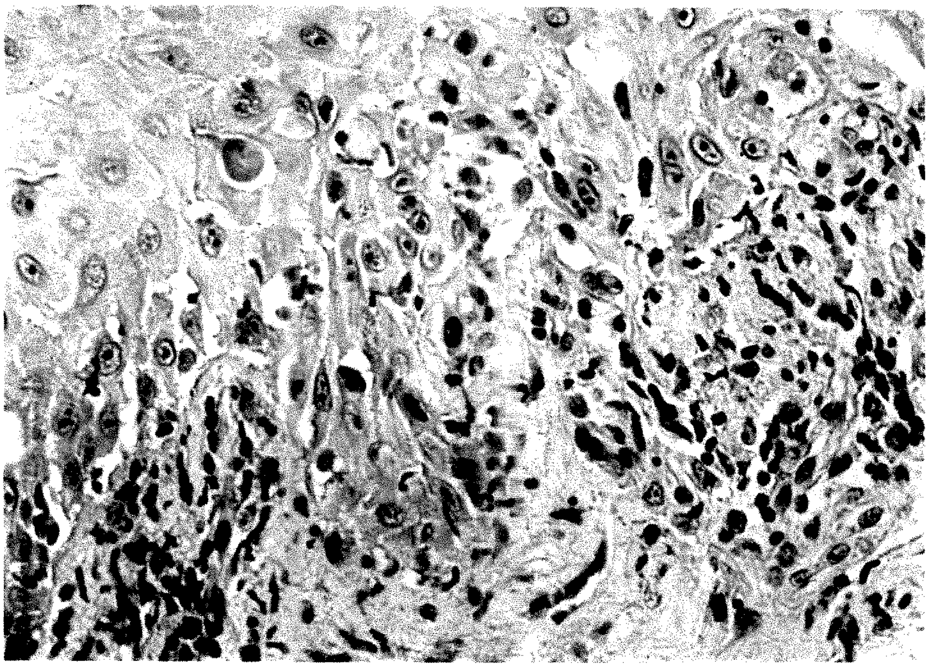

FIGURE 3. Higher magnification of the dermal–epidermal zone (H&E × 250).

Case III. A 20-year-old boy was admitted for idiopathic aplastic anaemia. Transplantation was performed with the marrow of his matched brother (HLA identical and non-reactive in mixed leukocyte culture). Extra dermatological data are reported in detail elsewhere (Gluckman *et al.*, 1975). Skin lesions appeared late after the transplant. On day 120, a slightly pruriginous papular erythematous follicular rash covered the neck and the trunk. Although resembling a follicular lichen planus, the lichenoid nature of this rash was assumed because a typical lichenoid appearance was seen on the inner surface of the cheeks (Plate 1e) and on the gums and because a skin biopsy showed a peri-pilar lichenoid pattern. The basal cell layer of epidermis and follicles showed focal cell necrosis and Civatte bodies. The hair follicle had a mononuclear infiltrate with Civatte bodies extending around its base. A keratin plug was present in its mouth. The rash faded after a 20-day course of prednisone (1/mg/kg/24 h) but a follicular hyperkeratosis persisted. The mouth remained unchanged. By day 300, all the lesions had disappeared.

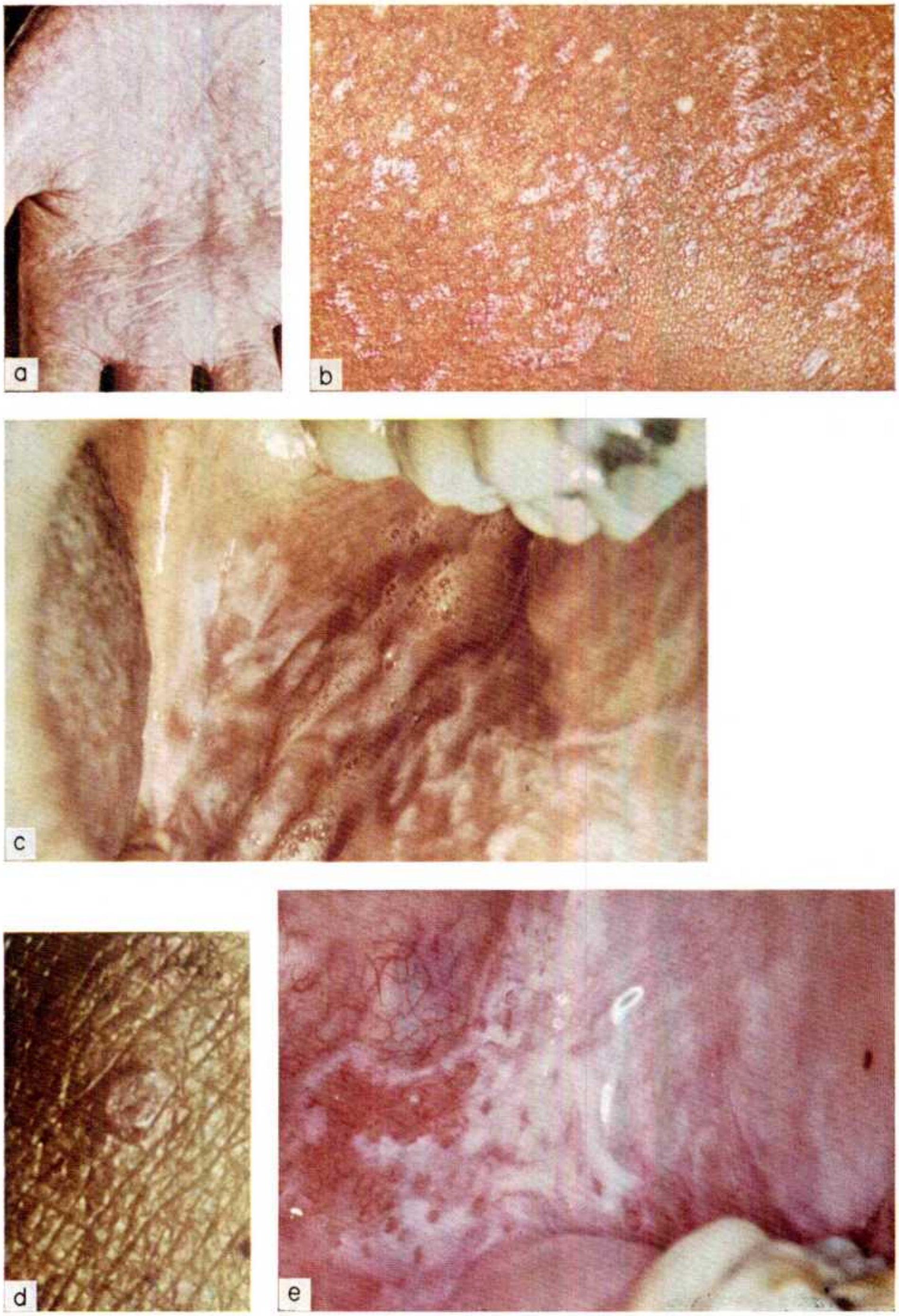

PLATE 1. (a) Lichenoid papules on the palm in case I, 80 days after the graft. (b) Lichenoid papules on the abdomen with a reticular pattern and deeply pigmented background; case I, 80 days after the graft. (c) Inner surface of the cheek in case II, 33 days after the graft. (d) One papule on the dorsum of the hand in case II, 45 days after the graft. (e) Inner surface of the cheek in case III, 130 days after the graft.

Case IV. A 10-year-old boy was admitted for acute lymphoblastic leukaemia. Transplantation was performed with the marrow of his matched mother (HLA identical and non-reactive in mixed leukocyte culture). In this case the lichenoid pattern was seen only on the inner surface of the cheeks and on the gums. This was first noted on day 35 and remained unchanged to day 130. On the skin a lichenoid appearance was never seen, the rash was only maculo-papular and hyperpigmented and was present from day 28 to day 50. Skin biopsy showed a grade II–III GVHR lesion (Lerner *et al.*, 1974). The patient remained severely immunodeficient and a *pneumocystis carinii* pneumonitis developed on day 110 which caused his death on day 130.

Correlation between the lichen planus-like eruption and the other clinical and biological signs

Out of the numerous clinical events and biological findings, only three biological parameters were found to be correlated with the occurrence or the relapse of the lichen planus-like eruption: an eosinophilia; a positive Coombs' test (complement); and the presence of anti-lymphocyte antibodies. The first two are interpreted as biological signs of GVHR; the third was reported recently in such a situation (Gluckman *et al.*, 1974). In our patients there was a clear correlation between the presence of such abnormalities and the lichenoid rash. Characteristically in case I they reappeared as the lichenoid rash relapsed on day 210; in case III they were first noted on day 110 and the lichenoid rash was obvious on day 120. It must be noted that the lichenoid rash in all four cases appeared when the blood lymphocytes were still non-reactive in mixed leukocyte cultures or when cultured with mitogens.

DISCUSSION

The lichenoid pattern was clinically obvious. Mouth involvement was very similar to lichen planus (Dr Kuffer, personal communication). Nevertheless the skin lesions, although lichenoid, were not always those of typical lichen planus (LP): case I showed a lichenoid erythroderma which occurs more often in lichenoid drug eruptions (Degos, 1953) than in LP. The reticular arrangement of the papules seen in case I is not a typical feature of LP. Involvement of palms and soles in LP is not always reddish-violaceous (Samman, 1972) as seen in case I. In case II, the few isolated papules on the dorsa of the hands more closely mimicked typical LP, as did case I on day 210 after grafting. It seems, therefore, that it would be better at present to consider these lesions as a lichen planus-like eruption (LPLE) instead of true LP.

Lichen planus-like eruption had not been reported in bone marrow transplant patients before our four cases and the case of Touraine *et al.* (1975). The reported skin lesions are described as maculo-papular or scarlatiniform rashes, or scaling and pigmented erythroderma (Mathe, Amiel & Schwarzemberg, 1965). Peck *et al.* (1972) have described toxic epidermal necrolysis in a patient with severe GVH reaction. These appearances seem to occur early after the bone marrow transplantation and our patients had initially maculo-papular rashes. Out of ten bone marrow transplant patients, four subsequently developed a LPLE; the six patients without LPLE died soon after the graft. Perhaps the LPLE outbreak is linked with a longer survival. However, LPLE has never been reported in long-term survivals after bone marrow transplantation (Slavin & Santos, 1973; Glucksberg *et al.*, 1974). It seems that a dermatologist had not managed these patients. The skin biopsy shown by Slavin & Santos (1973) is very similar to our LPLE biopsies but the clinical appearance is not described in dermatological words.

The histology was lichenoid, accounting for the clinical appearance as reviewed by Pinkus (1973); orthokeratosis, irregular thickness of the granular layer, acanthosis, basal cell necrosis, colloid bodies and pigmentary disturbances were present. The dermal infiltrate was not as intense as in typical LP but lymphopenia could account for this. Case I gave an interesting example of the histogenesis of the lesions. The biopsy of a maculo-papular lesion showed only basal cell damage. The second biopsy showed, besides basal cell damage, a slight acanthosis and hypergranulosis and at this time the clinical aspect was shiny, violaceous and reticular but slightly papular. As the clinical appearance became typically lichenoid, acanthosis was more marked and the dermal infiltrate was seen. It is obvious that basal cell layer damage preceded the dermal infiltrate as well as other epidermal changes. This is in agreement with Thyresson & Moberger (1957), Sarkany & Gaylarde (1970), and challenges Sabouraud's statement (1910). Basal cell layer damage appears thus as the first histological event. In our cases it is related to the graft-versus-host reaction (Lerner *et al.*, 1974).

The mechanism involved in this basal cell necrolysis has been reviewed by Peck *et al.* (1972). Merrit, Mann & Rogentine (1972) have found in the serum of bone marrow transplant patients an IgM antibody cytotoxic *in vitro* against tumoural epithelial cells. Cell mediated cytotoxicity could also be involved (Billingham & Streilein, 1968; Slavin & Santos, 1973). Whatever the nature (cell mediated, humoral, or both) of the immune reaction, such a mechanism probably accounts for the basal cell necrosis we observed in early skin biopsies in our patients. As basal cell damage is widely regarded as the first event in LP (Pinkus, 1973), occurrence of LPLE after an immune basal cell damage raises the question of the immune nature of this LPLE. This has to be critically examined.

The GVHR represents an acute rejection of the host organism by the transplanted immuno-competent allogenic cells. Symptoms occur usually in the first 20 days after the graft. Immunosuppression by drugs and anti-thymocyte sera can reduce the severity of this rejection, and the patient may survive. This treatment leads to a state of tolerance to recipient tissues by the transplanted cells; chronic immune reaction in addition to immunosuppressive drugs also leads to a severe immunodeficiency (Fass *et al.*, 1973). This promotes a high frequency of bacterial, fungal and viral infections. Symptoms of GVHR are then very difficult to differentiate from the symptoms of infection. This complex situation is called secondary disease (Mathe *et al.*, 1965) or chronic GVH disease (Thomas, 1974). It must be emphasized that LPLE has occurred in our patients during this chronic GVH disease. Even if the initial basal cell layer damage is most probably related to an immune reaction of immuno-competent allogenic cells against the recipient skin, the evolution throughout the lichenoid stage could be the result of a different event, for example modification of the epidermal cells by a virus or by a drug. Our patients have been extensively investigated for bacterial, fungal and virus infection. None seemed to be linked closely with the occurrence of LPLE. These findings do not exclude the role of a virus promoted by the immunosuppression. However, LPLE has never been reported after immunosuppression for kidney transplantation (Haim *et al.*, 1973). All the patients received various drugs before and after transplantation, none of which has been reported to cause LPLE (Bruinsma, 1973). The role of methotrexate (Pinkus, 1973) given just after the graft is unlikely as this drug was stopped 50 days before the beginning of the LPLE in case III and 150 days before its relapse in case I.

The hypothesis that LPLE could be a sign of chronic immune response against recipient epidermis seems more exciting. The clear correlation we found between the outbreak or the relapse of the LPLE and biological signs of GVHR (eosinophilia, positive Coombs' test and antilymphocyte antibodies) supports this interpretation. The working hypothesis for the study of LPLE after bone marrow transplantation seems thus to be that it is a chronic immune reaction involving the basal cell layer of the epidermis and leading to disturbances in the epidermal cell growth resulting in a lichenoid pattern.

Bone marrow transplanted patients are a 'man-made experimental model in man'. Will they become an experimental model of some aetiological aspects of lichen planus?

ACKNOWLEDGEMENTS

J-H.Saurat was the recipient of a grant of 'Fonds d'études du corps médical hospitalier de Paris'.

We thank the 'Direction régionale des Laboratoires UNILABO, Paris', for their financial support for the colour plates. This study was supported by I.N.S.E.R.M. grant no. 1975.42—A.T.P.

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Arterial thrombosis in scleroderma

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Accepted for publication 21 April 1975

SUMMARY

Clinical and pathological observations are presented on five patients with scleroderma who developed thrombosis of a major vessel. Three died following intestinal infarction or limb gangrene and two had digit or extremity amputations. Severe intimal thickening of major arteries with thrombus formation was noted but no significant abnormality was found in the clotting activity of the blood. Histochemical studies in three cases suggested a reduction of fibrinolytic activity at the site of thrombosis in the diseased vessels. These studies support the view that the vascular system is profoundly altered in scleroderma and may play an important role in some manifestations of the disease.

Progressive vascular disease is a striking feature of scleroderma. Distortion and obliteration of capillary loops and intimal proliferation in arterioles and small arteries are associated with such well-known clinical findings as skin telangiectasia, Raynaud's phenomenon, pulmonary hypertension and renal insufficiency. Much less common is massive and repeated thrombosis of major arteries which can cause considerable disability and even death.

Clinical and pathological data are reported on five patients with scleroderma in whom repeated thrombotic episodes in diseased arteries led to significant tissue destruction and gangrene. The coagulation mechanism was studied in three of these patients. Although no significant abnormalities were found in the clotting and fibrinolytic activities of the blood, an apparent reduction in fibrinolytic activity was observed at the site of thrombosis in the involved vessels.

MATERIALS AND METHODS

The records of 100 patients with scleroderma for the years 1960–1970 from the hospital files of Northwestern University Medical Center and the Abraham Lincoln School of the University of Illinois were reviewed to ascertain the frequency of vascular complications. Four patients had thrombotic occlusions of major arteries, with a fifth patient studied at the University of Chicago's Pritzker School of Medicine. The diagnosis of scleroderma was accepted if the patient had acrosclerosis and, in addition, either gastrointestinal involvement or sclerosis of the skin of the trunk and face, or involvement of

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the lung, heart or kidney. Gastrointestinal involvement was established by cineradiographic or manometric evidence of distal oesophageal hypoperistalsis or by radiographic evidence of colonic sacculations characteristic of scleroderma. Evidence for pulmonary involvement was based on radiographic demonstration of bilateral pulmonary fibrosis not caused by any other disease process, a clinical episode of pleurisy with pleural pain and rub or pulmonary hypertension with evidence of right heart failure. Cardiac involvement was defined as pericarditis with pain and rub, electrocardiographic evidence of pericarditis, or cardiomegaly with intraventricular conduction defect. Renal involvement was defined as rapidly progressive renal failure. Patients with other coexisting connective tissue syndromes or with organ involvement that could be attributed to some other concurrent disease process were not included.

Formalin-fixed tissue specimens obtained at surgery or autopsy were available for study on all five patients. All slides were re-examined and searched for arterial abnormalities.

Fresh tissue specimens obtained at surgery from two patients (cases 1 and 3) and at both surgery and subsequent autopsy from another patient (case 2) were studied by the histochemical fibrin slide technique as described by Todd (1959). The fresh specimens were snap-frozen in solid CO_2, embedded in OCT (Lab-Tek Instruments Co.) and stored at -70°C. Sections were cut at 6 μm with a Lab-Tek Cryostat microtome and collected on slides coated with a 0·06 mm layer of bovine fibrin, rich in plasminogen. As a control for nonspecific protease activity, the slides were also prepared with plasminogen-free bovine fibrinogen. After 1 min fixation in 50% methanol in saline, the slides were incubated in a moist chamber at 37°C for periods ranging from 3 to 60 min. After 10% formaldehyde fixation, the sections were stained with Harris' haematoxylin. Fibrinolytically active sites appeared as clear, punched-out zones of lysis in the opaque fibrin film (Todd, 1959; Kwaan & Astrup, 1967).

Plasma fibrinogen was assayed on citrated plasma by the method of Astrup *et al.* (1965). One-stage prothrombin time was performed using human brain thromboplastin as the coagulant (Biggs & Macfarlane, 1962) and partial thromboplastin time was performed by the method of Langdell (Langdell, Wagner & Brinkhous, 1953). Euglobulin lysis time was done by the method of Kowalski (Kowalski, Kopec & Niewarowski, 1959).

CASE REPORTS

Case 1. A 42-year-old white housewife developed Raynaud's phenomenon, sclerosis and telangiectasia of the skin of her hands, face and chest at the age of 30. At the age of 37, she developed severe pain and ulceration of her knuckles and finger tips. Radiographs of her hands showed resorption of the distal phalanx of her third left finger. Radiographs of her chest, oesophagus, stomach, small bowel and colon were normal.

At the age of 40, multiple ulcerations of her feet and ankles occurred. A femoral arteriogram showed occlusion of both popliteal arteries and her left foot and later her right foot became gangrenous, requiring below-the-knee amputations. The left stump failed to heal completely and because of progressive gangrene, an above-the-knee amputation was done. The right stump subsequently also became gangrenous and an above-the-knee amputation was performed. Microscopic examination of tissue obtained from these multiple amputation specimens showed severe sclerosis of the subcutaneous connective tissue with necrosis and atrophy of the skeletal muscle and necrotic bone and cartilage. Varying degrees of intimal hyperplasia, recent and old, with marked narrowing of lumina were noted in larger and medium-sized arteries. Evidence of recent thrombosis was seen in several of these arteries.

During her last hospitalization, when aged 42, clinical examination of heart, lungs and abdomen remained normal as were all routine laboratory studies, including renal function studies. Coagulation profiles, including prothrombin time, partial thromboplastin time, fibrinogen levels and euglobulin lysis time were normal. In spite of additional debridement, including a still higher amputation of the right thigh, gangrene of both stumps progressed. These debrided tissues again showed arteries with severe intimal hyperplasia. She died of sepsis without control of the relentless progression of gangrene. Autopsy was not obtained.

Case 2. A 55-year-old white female had Raynaud's phenomenon since early childhood. At the age of 42 she developed sclerodactyly, telangiectasia of the skin of her face, arms, trunk and buccal mucosa, and calcinosis cutis of her upper and lower extremities. Multiple areas of subcutaneous calcification, degeneration of the tufts of many of the terminal phalanges and fusion of the right carpal joint spaces were subsequently noted in hand X-rays. Later in the course of her illness, a dilated aperistaltic oesophagus was demonstrated by radiographic and manometric techniques and a moderate reduction of diffusing capacity was shown by pulmonary function studies. Renal function studies remained normal.

At the age of 50, necrotic skin ulcers and gangrene of the left foot occurred, requiring a below-the-knee amputation. Microscopic tissue examination revealed severe intimal fibrosis of major arteries with evidence of thrombosis and focal calcification. A recanalized thrombus was present in a large vessel and a thrombus was also seen in a small artery in the muscle. The myxomatous character of the intimal hyperplasia was more pronounced in veins than in arteries, where the thickening was more hyalinized. Two years later her right index and middle fingers were amputated because of gangrene. Microscopic tissue examination again showed multiple arteries with significant intimal hyperplasia. In one section, a subcutaneous artery was totally occluded by fibrous connective tissue.

She was hospitalized at the age of 55 because of persistent progressive ulceration and gangrene of her right foot. Fibrinogen levels, euglobulin lysis time, prothrombin time and platelet counts were normal. A below-the-knee amputation of her remaining leg was performed with microscopic tissue examination again showing intimal thickening and thrombosis in muscular and subcutaneous arteries. Two weeks after surgery she developed signs of intestinal obstruction and an exploratory laparotomy revealed thrombosis of the inferior mesenteric artery with infarction and perforation of the colon. Microscopically, the inferior mesenteric artery showed severe intimal hyperplasia with thrombotic occlusion of the lumen. Adventitial vessels were thickened, with marked intimal hyperplasia. She expired shortly after surgery.

At autopsy, arterial changes were present in numerous organs including the stomach, pancreas, periadrenal connective tissue, mesentery, intercostal muscle and connective tissue, the wall of the Fallopian tube and the pituitary stalk. These consisted of thickened vessels with hyalinized or fibrosed intima. Such changes were also noted in the wall of the ulcerated oesophagus, with a fresh thrombus in a companion vein. In the kidney, similar arterial changes were present but lacked the onion skin-like layering characteristic of scleroderma. In the lungs there were interstitial fibrosis and a hyaline membrane-like exudate suggestive of uraemic pneumonitis and focal areas of severe bronchopneumonia. The right coronary artery was severely atherosclerotic but there was no histological evidence of a myocardial infarction.

Case 3. A 27-year-old white female was well until the age of 23 when she noted Raynaud's phenomenon and acrosclerosis. Two years later her left third finger became ischaemic and a left thoracic sympathectomy was done. However, gangrene developed, necessitating amputation of the digit at the metacarpophalangeal joint. Tissue sections showed small arteries with marked intimal fibrosis in the subcutaneous tissue and one slightly larger artery with an old thrombotic occlusion and recent recanalization. An adjacent section of the same vessel was noted to have severe intimal hyperplasia. A right thoracic sympathectomy was performed later because of ischaemic pain in her right third finger.

The distal portion of this finger was later amputated. No subsequent episode of acute gangrene has occurred to date despite severe Raynaud's phenomenon.

During this time multiple telangiectases of the face and sclerosis of the skin of her hands, forearms and face have developed. An aperistaltic lower oesophagus was demonstrated by manometric studies. Other organ systems have shown no abnormalities. Coagulation studies, including fibrinogen levels, prothrombin time, partial thromboplastin time and euglobulin lysis time were normal.

Case 4. A 38-year-old white female was well until the age of 33 when she developed diffuse facial swelling followed by Raynaud's phenomenon, acrosclerosis and finger-tip ulceration. At the age of 37 she was hospitalized because of severe hypertension. On physical examination, sclerosis and multiple telangiectatic mats were noted on her face and forearms. Her blood pressure was 220/140 mm Hg. Examination of her eyes showed grade III hypertensive retinopathy. Her heart was enlarged and she had bilateral ankle oedema. Slight proteinuria was noted on examination of the urine. Abnormal swallowing complexes and an inadequate sphincter at the gastro-oesophageal junction were noted in oesophageal motility studies. A restrictive defect was found on pulmonary function testing. Her oedema improved with digitalis and antihypertensive and diuretic agents.

Several months later, severe cramping abdominal pain occurred. An upper gastro-intestinal X-ray showed a localized area of segmental narrowing in the small bowel, which proved to be gangrenous at laparotomy. A segmental resection with an end-to-end anastomosis of the remaining small bowel was performed. One year later, at the age of 38, severe cramping abdominal pain recurred. By the time she arrived at the hospital emergency room she was comatose and moribund. Laparotomy demonstrated gangrene of the entire small bowel, from the ligament of Treitz to and including the proximal portion of the ascending colon, due to thrombosis of the superior mesenteric artery. She died several hours after surgery.

At autopsy, the small intestine was necrotic. Sections of the superior mesenteric artery showed an old thrombus with recanalization and a superimposed recent thrombus occluding the lumen. Intimal fibrosis was seen in arteries of the kidneys, periadrenal fat, pancreas, adventitia of oesophagus, peritubarian connective tissue and in one artery in a portal area of the liver. The lungs showed severe intimal fibrosis of pulmonary artery branches, as well as pulmonary fibrosis. There was mild atherosclerosis of the aorta.

Case 5. A 43-year-old black female developed Raynaud's phenomenon at the age of 18, followed by sclerosis of the skin of her face, upper trunk and all four extremities. At the age of 35 she developed multiple finger-tip ulcerations. During the next 3 years, all digits of both hands were amputated either at the metacarpophalangeal joint or at the proximal interphalangeal joint because of gangrene. Microscopic examination of the arteries of the amputated digits showed partial or complete occlusion with medial and intimal thickening and thrombus formation.

At the age of 36, she developed left lower leg ulcers which progressed over 4 years to gangrene, requiring below-the-knee amputation. Tissue sections of the amputated lower extremity showed intimal hyperplasia of smaller arteries in the subcutaneous tissue, one of which showed a recent occlusion. The larger arteries and veins showed mild to moderate intimal hyperplasia, sometimes myxomatous in character. One artery was almost completely occluded by layered fibrous connective tissue in which there was focal calcification.

At present, she has large areas of ulcerated skin in her remaining foot. Mat telangiectases are present on the forehead and cheeks. Blood pressure is normal. Examination of the heart reveals a systolic grade II/VI ejection murmur in the tricuspid area and at the apex. Electrocardiogram shows evidence of left ventricular hypertrophy. Severe interstitial pulmonary fibrosis is visible in chest X-rays.

Oesophageal studies reveal aperistalsis of the lower oesophagus with a hypotonic lower oesophageal sphincter. The liver is enlarged 3 cm below the right costal margin. Renal function is compromised with 2 g proteinuria per 24 h and a creatinine clearance of 35 ml/min.

RESULTS

Clinical studies. Clinical data on the five cases are summarized in Table 1. All had Raynaud's phenomenon, acrosclerosis with finger-pad scarring and face and trunk sclerosis with mat telangiectasia. Four had persistent and severe digit ulceration, and digital tuft resorption on X-ray. Large leg ulcers were predominant clinical features in three patients. Four of the five had cineradiographic and/or manometric evidence of oesophageal hypoperistalsis. Pulmonary function tests in three of four patients showed decreased diffusion capacity. One patient (case 5) had abnormal renal function and another (case 4) mild proteinuria without progressive renal disease. In the remainder, no evidence of renal disease was present. Except for the development of large vessel thrombosis, these patients did not appear to be clinically different from other individuals with scleroderma.

TABLE 1. Clinical and laboratory findings in patients with progressive systemic sclerosis who had large and medium vessel thrombosis

	Case				
Symptom	1	2	3	4	5
Raynaud's phenomenon	+	+	+	+	+
Acrosclerosis	+	+	+	+	+
Finger-tip ulceration	+	+	+	o	+
Digital tuft resorption	+	+	+	ND	+
Face and trunk sclerosis	+	+	+	+	+
Telangiectases	+	+	+	+	+
Leg ulceration	+	+	o	o	+
Oesophageal hypoperistalsis	o	+	+	+	+
Abnormal pulmonary function	ND	+	o	+	+
Abnormal renal function	o	o	o	+	+
Hypertension	o	o	o	+	o

+ = present; o = absent; ND = not determined.

Histological studies. All five cases demonstrated the vascular alterations described in patients with scleroderma (D'Angelo *et al.*, 1969). Myxomatous, or sometimes more hyalinized, intimal proliferation was noted in arteries of various sizes. This proliferation was usually circumferential but was also focal. In some vessels the entire lumen was occluded. Veins were affected similarly but usually less severely. Occasional focal calcium deposits were present, with blood vessels in upper and lower extremities and a variety of visceral organs being involved. In one instance, intimal changes in pulmonary vessels were associated with severe pulmonary fibrosis. The myxomatous type of arterial change that has been associated with kidney involvement was absent, as might be expected, in the kidneys of the two patients (cases 2 and 4) who lacked evidence of renal insufficiency. Renal tissue was not available from the other patients including case 5, who had clinical evidence of renal function impairment. Clearly, the organ distribution of these arterial changes varies from one case to another and determines the major clinical manifestations.

In addition to these vascular changes characteristic of scleroderma, fresh or recanalized thrombi were observed superimposed upon areas of intimal thickening in vessels adjacent to areas of gangrene. These thrombotic changes were thought to be the cause of the ischaemic event rather than its consequence. It seems unlikely that any single thrombosis was the result of contiguous chronic inflammation since arterial tissue was examined from sites remote from tissue necrosis or inflammation.

Blood coagulation studies. Fibrinogen levels, prothrombin time, partial thromboplastin times and euglobulin lysis time were within normal limits in the three patients studied.

Tissue fibrinolytic studies. The fibrin slide technique was used to study vessels obtained at surgery from three patients (cases 1, 2 and 3) and at autopsy from one patient (case 2).

In apparently unaffected vessels of kidney, spleen and lung without intimal proliferation or narrowing (case 2), clear zones of lysis were seen easily at the endothelial cell layer, indicating normal fibrinolytic activity (Fig. 1). Many smaller vessels and capillaries in these sections also showed similar activity.

Examination of diseased vessels with intimal proliferation and fibrosis but without evidence of old or fresh thrombi in their lumens revealed variable lytic activity. Most of the small arteries in the dermis, subcutaneous tissue and muscle in sections from cases 1, 2 and 3 had active fibrinolysis. Normal fibrinolytic activity also was observed in arterioles and small arteries in the spleen and

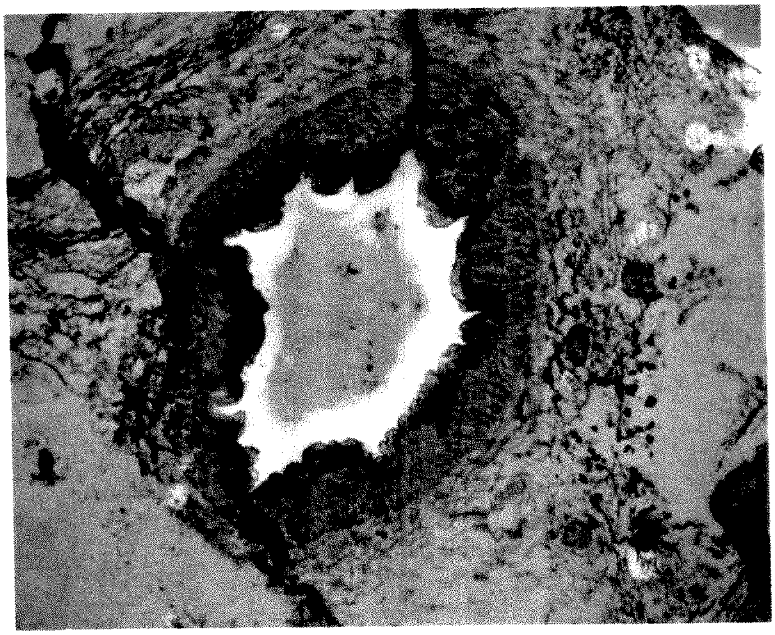

FIGURE 1. Normal vessel (case 2) shown by the fibrin slide technique after 30 min incubation. Shows clear zones of lysis along the endothelial layer at sites where fibrin has been digested. Similar activity is seen in many small capillaries in the adjacent tissue. Harris alum haematoxylin stain.

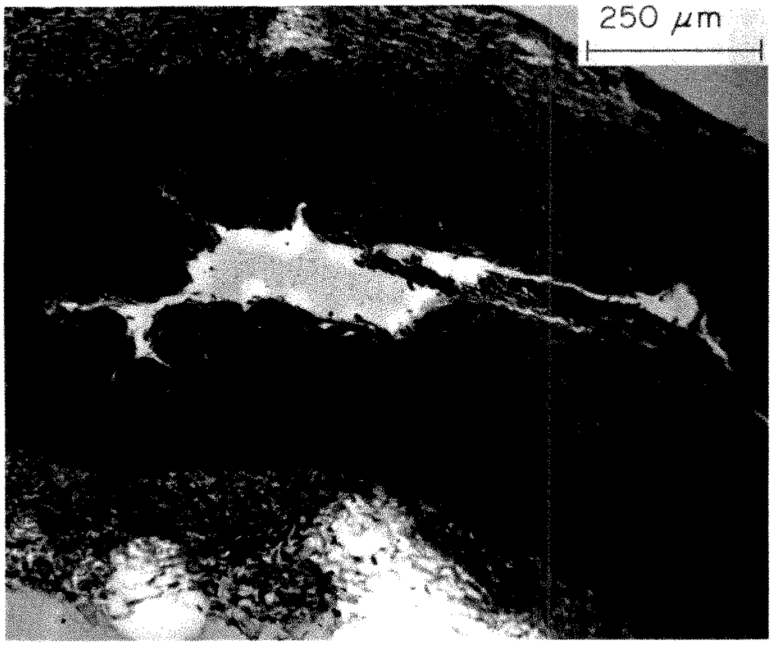

FIGURE 2. Mesenteric artery (case 2) showing a small mural thrombus adherent to the thickened intima. No evidence of lysis is present at the site of thrombus attachment, although lytic zones are present in areas of intimal proliferation away from the thrombus as well as in small capillaries in the surrounding tissue, seen after 45 min incubation with the fibrin slide technique. Harris alum haematoxylin stain.

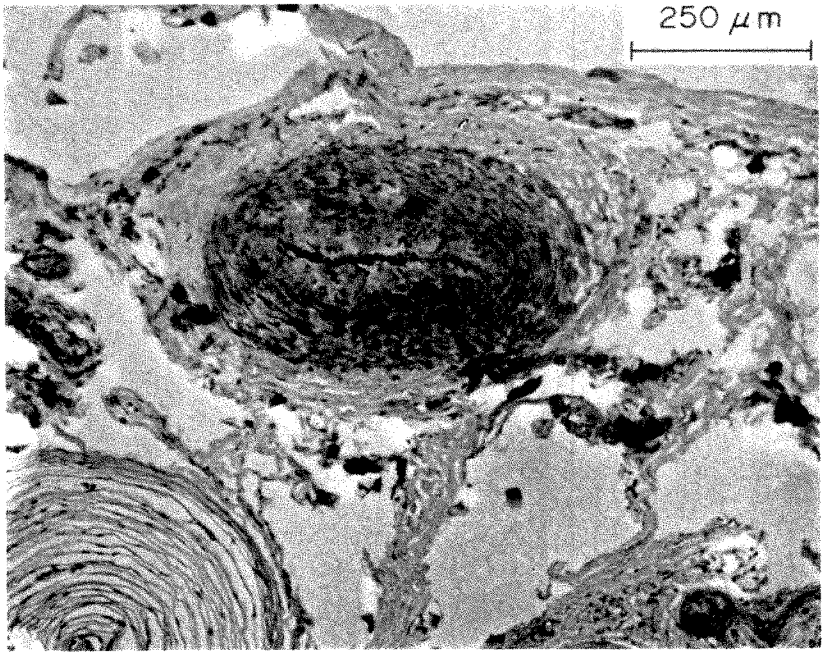

FIGURE 3. Digital artery (case 3) has no evidence of lytic activity at the site of a recent thrombus which fills the slit-like lumen. Lytic activity is present in capillaries in surrounding tissue, seen after 45 min incubation with the fibrin slide technique. Harris alum haematoxylin stain.

mesentery with intimal proliferation and fibrosis but without thrombi. However, several smaller arteries from subcutaneous tissue in case 3 failed to show activity at the intimal border.

In contrast, those vessels with thrombosis superimposed upon intimal proliferation showed complete absence of fibrinolytic activity. This is illustrated in a section of mesenteric artery of case 2 where a small mural thrombus is adherent to the markedly thickened intima (Fig. 2). Lysis is absent at the site of the thrombus, although lytic zones are present in areas of intimal proliferation away from the thrombus. In a small artery from the amputated digit in case 3 there is intimal thickening, the endothelium is not recognizable and a recent thrombus fills the slit-like lumen. No lysis is seen in the lumen of this vessel (Fig. 3).

COMMENT

In patients with progressive scleroderma, abnormalities in the microcirculation have been well documented. Telangiectatic macular skin lesions and nailfold capillary changes are frequently noted (Kogoj, 1967; Maricq & Le Roy, 1973). Norton *et al.* (1968) reported an overall reduction in the number of capillaries in skeletal muscle with many of the remaining capillaries showing thickening and reduplication of the basement membrane and degeneration of the endothelium. Schumacher (1972) found obliteration of vessels in synovial tissue and in remaining vessels and noted multiple layering of the basement membrane and heavy infiltration of vessel walls with fibrin. LeRoy, Downey & Cannon (1971) demonstrated that skin capillary blood flow is reduced during a cooling stimulus but returns toward normal with warming. However, even on warming, the skin temperature remained lower than in normal subjects, indicating permanent constriction of some vessels.

Arterial changes too, are well recognized, particularly in the smaller vessels. Raynaud's phenomenon is found in over 90% of patients. Partial anatomical occlusion of the digital artery lumen occurs in addition to the reversible vessel contraction induced by exposure to cold (Lewis, 1938; Dabich *et al.*, 1972). Arterial involvement of lungs and kidneys as well as other tissues has been repeatedly documented. The characteristic vascular lesion is proliferation of a loose myxomatous connective tissue within the lumen of the vessel. Concentric fibrosis may follow with narrowing and even obliteration of the lumen. Similar changes may be observed in veins.

Thrombosis of major arteries is a much less commonly recognized complication of scleroderma. As early as 1924, Matsui (1924) in a clinical and pathological description of six patients described two whose digital arteries showed intimal thickening with organizing clots. Lewis (1938) observed organizing thrombi in the lumen of digital arteries of two patients with scleroderma as well as several with severe Raynaud's phenomenon. Lushbaugh, Rubin & Rothman (1948) reported the first fatal complication of vascular disease of the colon in a patient with scleroderma. They postulated that the intestinal mucosa was devitalized by the obliterative process in the small arterioles resulting in trophic ulceration and necrosis with widespread thrombosis and peritonitis.

There have been additional case reports of infarction and gangrene of extremities and viscera (McMahon, 1972; Toth & Alpert, 1971; Barnett & Coventry, 1969; Tay & Khoo, 1970; Edwards & Lennard-Jones, 1960) but definite thrombotic occlusion was not implicated. In some cases, the gangrenous tissue may not have been extensively examined for evidence of thrombosis. However, in the case reported by McMahon (1972), extensive dissection of the infarcted intestinal tract and liver was done and no thrombus was found. It was postulated that vasospasm was the cause of the massive necrosis and that the diffuse underlying sclerosis and intimal thickening of small vessels was the precipitating factor initiating the vasospasm.

The thrombotic events that have been described, both in the literature and in our study, appear to be directly related to the underlying arterial and venous disease of scleroderma rather than to the coexistence of an unrelated process such as atherosclerosis. Atherosclerosis of this severity does not usually affect this younger and predominantly female population who developed thrombosis. Only mild atheromatous changes were seen in cases 2 and 4. Nevertheless, one cannot entirely exclude the related possibility that atherosclerotic disease might occur prematurely and superimpose itself as a thrombotic disease upon the already damaged vasculature of scleroderma. Medsger & Masi (1971) have shown that older scleroderma patients have a greater risk of cardiovascular disease, suggesting the possibility that concomitant atherosclerotic disease may be favoured in this disorder.

A generalized disorder of the clotting mechanism was not documented in our group. The standard parameters of clotting and fibrinolysis were normal in these patients and in several other scleroderma patients without thrombosis who were tested. In contrast, a diminished or absent fibrinolytic activity was shown in the endothelium of some arteries that had undergone intimal connective tissue proliferation. Since an active fibrinolytic system is required for the removal of the normal amounts of fibrin deposited on endothelium to repair small defects (Astrup, 1969), compromise of this mechanism may result in unchecked thrombus formation within the vessel lumen in a manner similar to that which has been described in thrombotic thrombocytopenic purpura (Kwaan *et al.*, 1968). Limitation of fibrinolytic activity may be further compromised by inhibitors of fibrinolysis present within platelets that are regularly aggregated within arterial thrombi (Kwaan & Suwanwela, 1971).

Although such a sequence of events might account for the thrombotic events observed in our patients, it cannot be accepted without several reservations. The tissue fibrinolytic test system is qualitative and does not provide an exact measurement of the degree of fibrinolysis. Nor does it show the rate of synthesis of activator material but only indicates the presence of activator material that is preformed within the tissue specimen and available at the time of testing. Also, our observations were confined to limited amounts of tissue and may not reflect the total pathology of the lesion. Finally, the conclusion that the fibrinolytic defect precedes rather than follows thrombosis is based on the observation that fibrinolysis was impaired in some non-thrombosed vessels with proliferative intima.

The pathogenesis of scleroderma has been the subject of speculation, study and controversy for many years. Dinkler (1891) postulated that the alterations in connective tissue reflect underlying vascular disease. This view, suppressed for many years, was revived by Ingram (1958) and more recently by Norton & Nardo (1970). Summarizing a large body of evidence, they proposed that scleroderma 'be classified as a vascular disease, and the primary site of injury is at the microvascular level'.

An opposing view emphasizes changes in connective tissue. Unna (1896) first proposed the view that capillary obliteration in the skin is the result of fibrosis of connective tissue. Many authors have endorsed this view. Abnormal connective tissue has been observed in the dermis by electron microscopy (Fleischmajer, Damiano & Nedwich, 1971). Increased collagen biosynthesis, as measured by protocollagen proline hydroxylase activity, was reported by Uitto, Hannuksela & Rasmussen (1970). LeRoy (1972) found increased synthesis of soluble collagen and glycoprotein fractions by scleroderma fibroblasts in cell culture, while chondromucoprotein levels were decreased. Sclerosis of connective tissue would seem to be a result of an abnormal function of fibroblasts.

The observation of endovascular proliferation and sclerosis, independent of encroachment by surrounding fibrous tissue and the possible decrease in fibrinolytic activity in arterial endothelium, suggests that alterations of endothelium generally, and not only in capillaries, play a role in scleroderma. One may view the fibroblast and the endothelial cell as first cousins within the connective tissue family, with many similarities in morphology and function, obvious differences in both spheres notwithstanding. Both cells produce collagen and mucosubstances. A similar derangement in the function of these two cells leading to the abnormal secretion of normally produced substances could explain both connective tissue and arterial manifestations of scleroderma.

ACKNOWLEDGEMENTS

We are grateful to Dr Alan Lorincz and Dr Nancy Esterly for their permission to study cases 2 and 5 respectively, and to Dr C.A.Krakower, Dr R.Wissler and Dr J.Sherrick for their permission to review pathology records of the various patients.

The project was supported by grants from the USPHS, AM 11513, Illinois Chapter, Arthritis Foundation and the Jordan Listick Research Foundation.

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British Journal of Dermatology (1975) **93**, 695.

Case Reports

Human infestation by *Ophionyssus natricis* snake mite

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Accepted for publication 14 March 1975

SUMMARY

A family presented with a papular vesiculo-bullous eruption of the skin, found to be caused by the snake mite, *Ophionyssus natricis* (Gervais, 1844). A pet python was the primary host. Treatment of the animal and its environment led to clearance of the human skin lesions.

Mrs A.J., aged 37 years, and her three children, aged 3, 7 and 9 years, complained of an itchy papular eruption of 5 months' duration. The onset of the symptoms had commenced with the family adopting a 2 metre long pet python. The mother presented with a papular rash on the forearms (Fig. 1) but the children, whose pet it was, frequently carried the snake around their necks and bodies and even took it to bed with them, and they presented with much more widespread lesions, many of which revealed black central dots due to skin puncture and bleeding; some of the lesions were vesicular or indeed bullous. A biopsy from one of these revealed a large subcorneal bulla with an exudate of polymorphonuclear leukocytes; the basal layer revealed some vesiculation and there was severe subepidermal oedema.

The family had noticed 'small insects' fixed on their skin and which they had attempted to remove by needles; they had also observed large numbers of these 'same insects' on the snake, particularly around its head, and in the chair which it usually occupied. One of these mites was identified as the Dermanyssid snake mite, *Ophionyssus natricis* (Gervais, 1844).

The snake and its cage and chair were liberally dusted with Pyrethrum powder. There were no further complaints from the family.

Notes on Ophionyssus natricis *(Gervais,* 1844*) Fig.* 2

This species is a purely blood-sucking parasite of captive snakes, and occasionally lizards, and is of world-wide distribution. The weight of an adult female may increase 15 times during a single blood meal and a heavy infestation of several hundred mites may therefore lead to severe anaemia, listlessness, refusal to eat, and death of the host snake. *Ophionyssus natricis* is also known to transmit *Proteus hydrophilus*, an organism capable of producing a fatal haemorrhagic septicaemia in snakes.

Unfed females are about 600 μm in length and yellowish brown, and fully engorged may be 1300 μm in length and dark red or black. *Ophionyssus natricis* is described more fully by Baker *et al.* (1956). The life cycle consists of five developmental stages: egg, larva, protonymph, deutonymph and adult. The complete cycle from egg to ovipositing adults probably takes 13–19 days, and the mite may live as

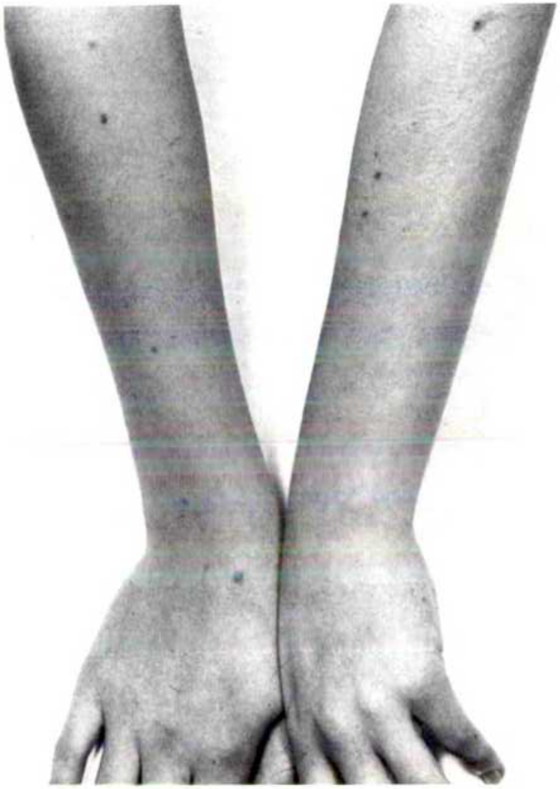

FIGURE 1. Papular eruption on forearms.

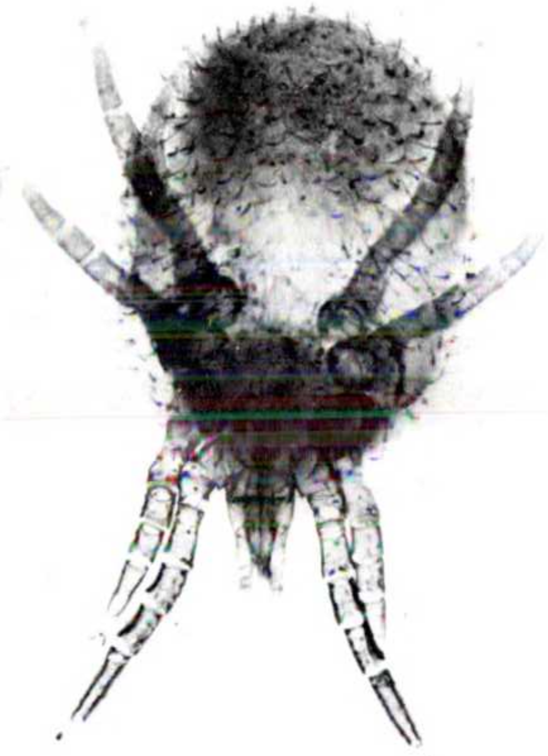

FIGURE 2. *Ophionyssus natricis.*

long as 40 days. An adult female feeds 2 to 3 times, and after every blood meal she leaves the host and deposits a batch of eggs in crevices or rough shady places. One female may deposit up to 80 eggs and a blood meal is necessary for each nymphal stage of development.

Pet animal infestations are a common cause of human skin eruptions (Hewitt, Walton & Waterhouse, 1971). Snakes are uncommon pets but their popularity is increasing. Although not previously reported in the dermatological literature, *Ophionyssus natricis* should be considered when patients with unusual pets present at clinics.

ACKNOWLEDGMENTS

I am indebted to Professor Schmidt, University of Odense, for permission to publish this case, to Professor Niels Haarlos for identification of the mite and to Dr Mark Hewitt for help and encouragement in the preparation of this case report.

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British Journal of Dermatology (1975) **93**, 699.

Keratoacanthoma in a Bantu patient

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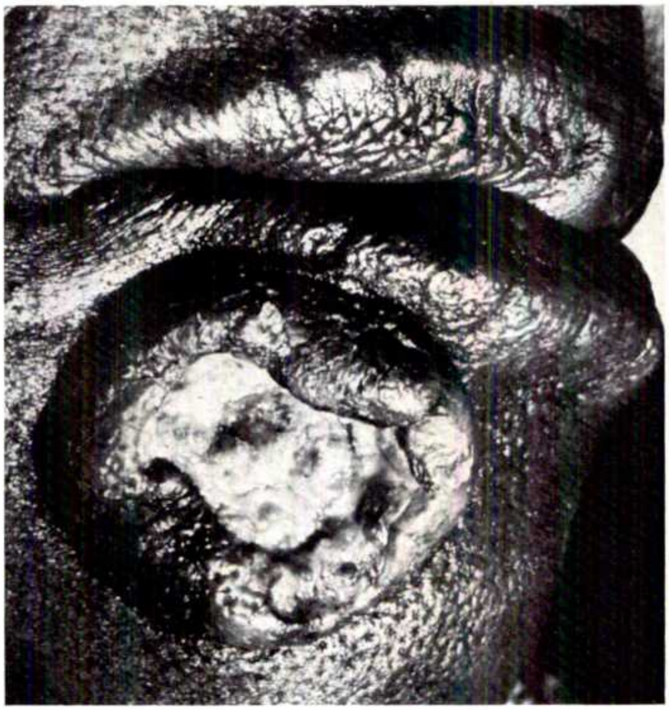
Accepted for publication 14 March 1975

Keratoacanthoma in the white Caucasian is common enough and all varieties are recognized. It is however extremely rare in the Negro races, and has for instance been diagnosed histologically only 4 times in 2 years at the South African Institute for Medical Research at Johannesburg out of a total of 1103 benign tumours seen during that period, and even these four cases were open to doubt as clinical information was not available (Rippey & Schmaman, 1972). The following patient therefore warrants reporting.



CASE REPORT

A 68-year-old black South African woman presented at the Dermatology Out Patient Department with a 3 weeks' history of a painless, rapidly growing tumour on the chin. She was otherwise quite well.

Examination revealed a tumour approximately 3 cm in diameter with raised borders and a central crateriform ulcer very suggestive of keratoacanthoma (Fig. 1). Punch biopsy revealed squamous hyperplasia but the pathologist could not distinguish between a well-differentiated squamous carcinoma and keratoacanthoma. Two weeks later, resection of the tumour was performed and the specimen submitted for histology. Comparison with the clinical photograph showed the lesion to have shrunk in size considerably and on histological examination of serial sections no evidence of neoplastic squamous epithelium could be found, although small islands of squamous epithelium in the process of being resorbed were seen.

Marshall (1964) stated that he was not aware of any case of keratoacanthoma in a Negro patient in which the history, clinical findings and pathological change all went to confirm the diagnosis. Our patient meets all these requirements and must be considered one of the very rare instances of keratoacanthoma in a black patient.

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Angioma serpiginosum—familial incidence

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Accepted for publication 24 March 1975

SUMMARY

In this paper we present the first record of familial incidence in angioma serpiginosum observed in two families. The clinical and histological features are described.

Angioma serpiginosum is an uncommon dermatosis, described by Hutchinson in 1889 and named by Crocker in 1894. The condition, commoner in females, starts before puberty as pin-sized capillary puncta affecting any part of the body except the mucous membranes, palms and soles. Progression is by the development of satellite punctate lesions; regression is rare; there is no background erythema and no pigmentation.

The rarity of the condition and the lack of diagnostic criteria has in the past led to its inclusion amongst the pigmentary purpuric dermatoses; however the two conditions were clearly separated by Barker and Sachs in 1965.

Four cases of a vascular naevus diagnosed as angioma serpiginosum with a familial incidence are described.

CASE REPORTS

Case 1

M.R., an 11-year-old schoolgirl (III.8 in the pedigree of family 1), presented aged 4 with 1 year's history of a rash on both legs. After 7 years' observation the rash has spread from both calves up to the thighs and down to the ankles. It has caused no symptoms.

There is no significant past medical history. Both parents are Spanish. There is a family history of a similar rash on the paternal side; the father, aunts, uncles and cousins are affected as shown in the pedigree of family 1 (Fig. 1).

On examination there is a diffuse punctate serpiginous eruption, flat, and red-purple in colour, extending from both thighs to the ankles (Fig. 2). It is most marked on the left calf. There was some blanching to diascopy but no clinical pigmentation.

Case 2

E.M., the 36-year-old aunt of case 1 (II.2 in the pedigree of family 1), first developed a rash on both

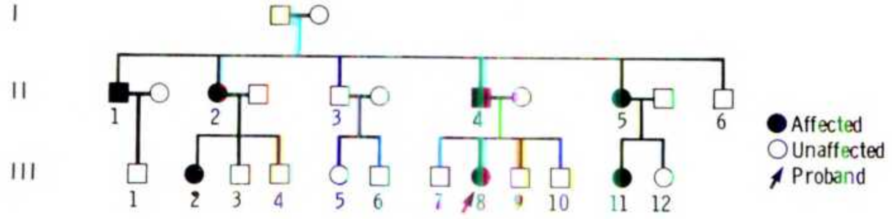

FIGURE 1. Pedigree of family 1.

legs at the age of 4. It has always been extensive and a constant cosmetic embarrassment. At the age of 12, areas of skin affected by the rash were removed in Spain in an attempt to prevent the rash from spreading. There is no other significant medical history.

Examination reveals an eruption similar to case 1. Typical punctate lesions are most marked around the ankles. They blanch partially on pressure. There are more diffuse fainter punctate lesions from the calves up to the thighs.

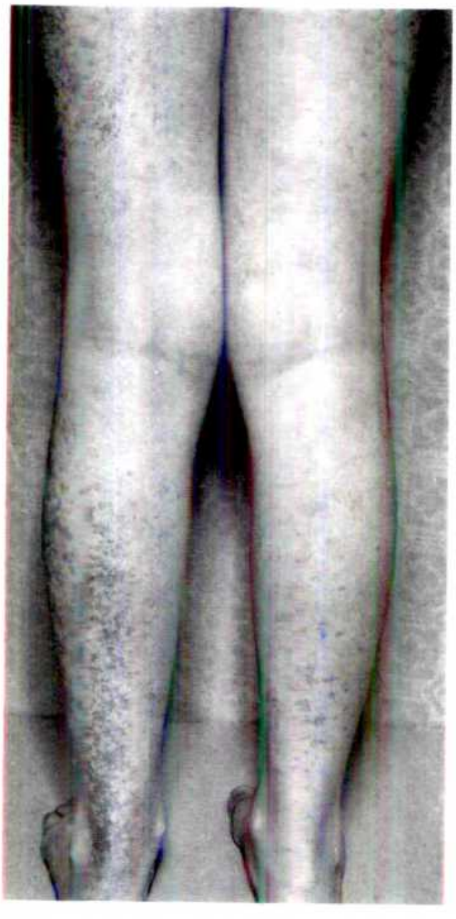

FIGURE 2. Case 1. The typical distribution of the rash on the calves and thighs.

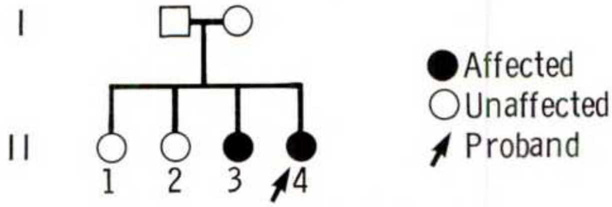

FIGURE 3. Pedigree of family 2.

Case 3
M.M., the 8-year-old daughter of case 2 and first cousin of case 1 (III.2 in the pedigree of family 1), developed a red pin-point rash over both calves over the course of a year. There is no other significant medical history.

Examination reveals the same red-purple punctate lesions on both calves slightly asymmetrical, being more marked on the left. It blanches partially to pressure.

Case 4
N.D., a 12-year-old school girl (II.4 in the pedigree of family 2), developed a rash on the right calf at the age of 18 months. It remained static until she was 6 when, her mother reports, it began to enlarge by the appearance of red dots at the periphery. At the age of 6 the patient suffered recurrent epistaxes necessitating cautery of the nasal septum. The patient's 14-year-old sister has had a similar rash on the right thigh since the age of 11.

Examination reveals a red-purple serpiginous eruption extending from the inner aspect of both thighs down to the ankles of both legs. A bilateral equinus deformity of both feet is present.

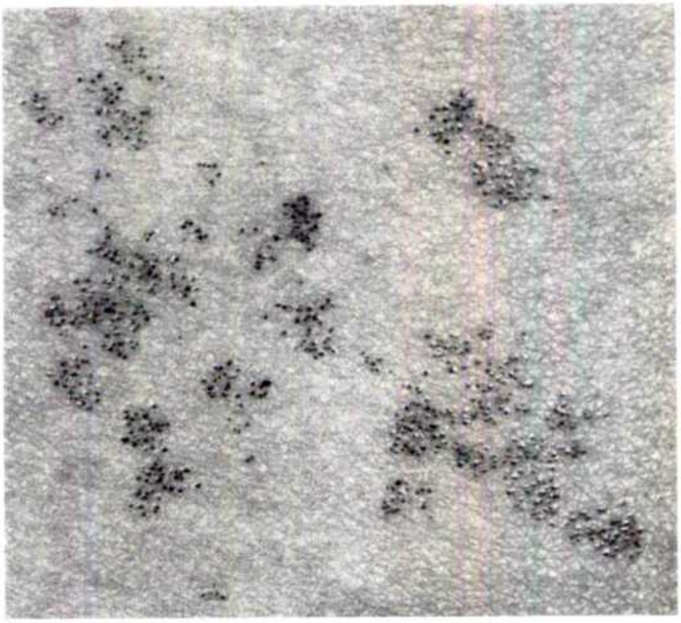

FIGURE 4. Close-up of the rash showing groups of individual punctate lesions.

Summary of clinical features

The typical lesions well shown in Fig. 4 are diffuse, punctate and blanch partially on diascopy. The arrangement of the red spots at the periphery has the appearance of streaks where new lesions develop unevenly. This gives the serpiginous appearance. There is no background erythema and no clinical pigmentation. The majority of the lesions are flat; only the occasional one is palpable.

Histology

3 mm punch biopsies of typical lesions were taken from cases 1, 2 and 4. They were fixed in formalin, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin.

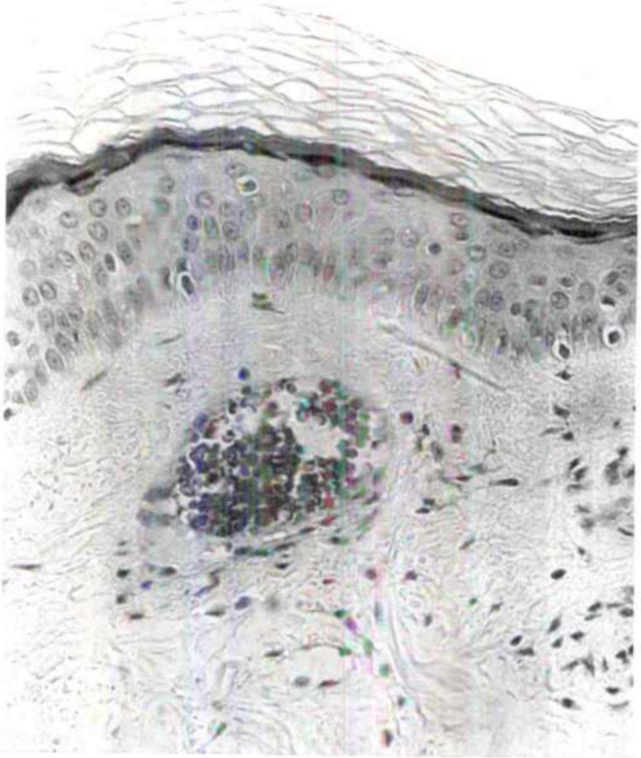

FIGURE 5. Histology of a typical lesion. Stained with haematoxylin and eosin. There is a single telangiectatic vessel lined by a single layer of endothelium (Magnification × 300).

Histologically the appearances are of telangiectatic blood vessels in the superficial papillary dermis. Each vessel is thin walled with no endothelial proliferation. They occur singly. Each vessel probably represents a single punctum observed clinically (Fig. 5).

DISCUSSION

The eruption in these four patients is typical of angioma serpiginosum, well described in the papers of Barker & Sachs (1965) and Frain-Bell (1957).

All our reported cases are females. Only two males, II.1 and II.4 in the pedigree of family 1 are reported by relatives to have the condition. 90% of Frain-Bell's cases (1957) occurred in females.

Although all parts of the body with the exception of mucous membranes have been reported to be affected, the condition has a predilection for the lower limbs. In all our patients the lesions are confined to the legs. In half of Frain-Bell's series of thirty only the legs were affected. Barker and Sach's case had the rarely described involvement of the soles of the feet.

The age of onset varies widely. Barker and Sach's case began at the age of 27. However all Frain-Bell's cases developed before the age of 14 and in his review of the British literature all nineteen cases started before the age of 22. Our four cases all began before the age of 10.

Slow progression is the usual course. Regression is unusual. However a cousin of cases 1 and 3, namely III.2 in the pedigree of family 1, cleared spontaneously at 12 years of age.

Histologically there is only the occasional telangiectatic blood vessel with no evidence of vasculitis or extravasation.

The purpuric pigmentary dermatoses are commonly maximal in dependent areas. There may initially be a punctate purpuric appearance but extravasation and later pigmentation occur. Majocchi's disease is dominated by inflammatory cells around dilated superficial blood vessels; Schamberg's disease is characterized by macular hyperpigmentation; while Gougerot and Blum's disease shows lichenoid changes clinically.

A number of familial angiomatous disorders are recognized. The 'port wine stain' (naevus flammeus) shows a familial incidence and dominant inheritance is suggested (Rook, 1972). The 'strawberry naevus' (cavernous naevus) is often described in several members of one family, 60–70% being female.

Cutaneous haemangiomata in association with systemic angiomata are more commonly recognized to have a genetic basis. The blue-rubber-bleb naevus syndrome has been suggested from two pedigrees to be an autosomal dominant with increased male penetrance (Berleyne & Berleyne, 1960; Talbot & Wyatt 1970; Walshe, Evans & Warin, 1966). The von Hippel–Lindau and Osler–Weber–Rendu syndromes are generally thought to be autosomal dominant conditions.

Familial incidence has also been described in macrocephaly and pseudopapilloedema in association with multiple cutaneous haemangiomata (Riley & Smith, 1960), and in Maffucci's syndrome (Bean, 1958).

Angioma serpiginosum should therefore be regarded as an uncommon capillary naevus syndrome which usually occurs sporadically but may occur with a familial incidence. No systemic manifestations have been demonstrated. The incidence in the first family we describe is consistent with autosomal dominant inheritance with variable penetrance. Penetrance is higher in females. Family 2 would also be consistent with this explanation although recessive inheritance cannot be excluded. A dominant gene might also explain the sporadic cases described in the literature, many of which could have occurred in new mutations.

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British Journal of Dermatology (1975) **93**, 707.

Hypertrichosis due to diazoxide

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Accepted for publication 26 March 1975

SUMMARY

Two cases of hypertrichosis due to diazoxide are described. The mechanism is unknown but may be due either to increased cutaneous perfusion or to increased levels of cyclic AMP.

Diazoxide (Endemine) is a hypotensive benzothiadiazine which causes hyperglycaemia, due in part to an inhibition of insulin secretion. For this reason it is recommended for the treatment of idiopathic hypoglycaemia of infancy (Drash *et al.*, 1968). It is rarely indicated in the conventional treatment of hypertension, but it is occasionally given for hypertensive nephropathy and malignant hypertension (Pohl & Thurston, 1971; Mroczek *et al.*, 1971). Hypertrichosis is a distressing side-effect of this treatment which does not seem to have been reported in the dermatological literature, and two cases are described here.

Case 1

P.C., now a 9-year-old boy, developed convulsions at the age of 3 months due to hypoglycaemia. At the age of 6 months treatment with diazoxide was started and this has been continued in a dose of about 250 mg twice daily until the present time. His mental and physical development have been slow; in 1973 his I.Q. was 62. In the first 5 years of treatment with diazoxide he had only two hypoglycaemic convulsions, but recently these have become more frequent, and he now has four or five such episodes each year. Investigations have shown inappropriate insulin secretion and a partial pancreatectomy was performed in March 1974, since when he has also been treated with chlorothiazide 250 mg at night and with potassium supplements. His general health is otherwise good.

His parents noticed the widespread development of blonde downy hair within a few months of starting treatment with diazoxide. This was most noticeable on the extensor aspects of his arms, chest and forehead and it became progressively more severe as time went by (Figs 1 and 2). The longest abnormal hair is now to be found on the arms where it is about 3 cm long, and shows intermittent medullation and some pigmentation. It is, therefore, more like terminal hair than vellus hair. The hair from the back, by contrast, has the characteristics of vellus hair, being fine and non-medullated, with very little pigment. Scanning electron microscopy of the hair from both these sites showed the surface microanatomy of the shafts to be normal.

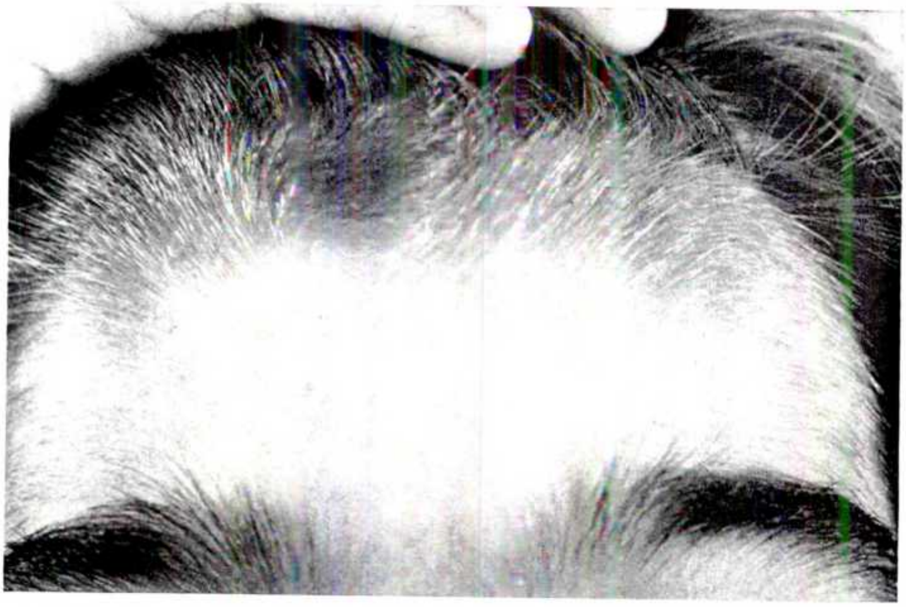

FIGURE 1. Case 1, aged 9 years, showing the marked hypertrichosis of the forehead.

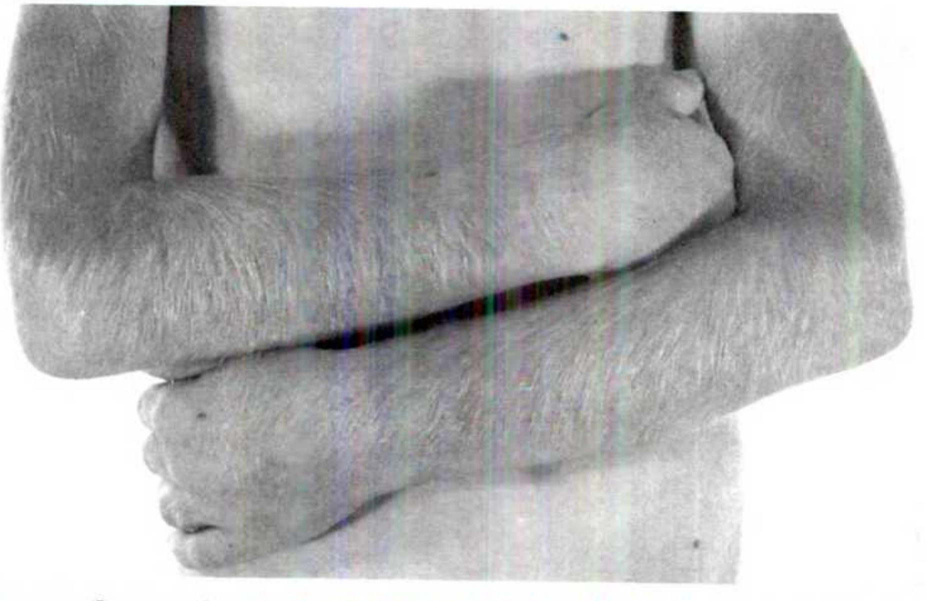

FIGURE 2. Case 1, aged 9 years, showing the profuse growth of blonde hair of the intermediate type on the arms, with sparing of the anterior trunk.

Case 2

E.C., now an 80-year-old woman, developed severe hypertension in 1956 and was treated in the following years with a variety of hypotensive drugs including methyldopa, debrisoquine, propranolol and clonidine. These drugs produced various side-effects and in August 1972, diazoxide (50 mg four times daily increasing to 100 mg four times daily) was substituted with good effect. By November 1972, she was noted to have hyperglycaemia with glycosuria, facial hypertrichosis, dryness of the skin with eczematous changes on the arms, and brown pigmentation of the face and hands. The hypertrichosis, which consisted of fine black hairs, involved the whole face but was particularly prominent over the upper lip, malar area and around the eyebrows (Fig. 3). The patient found the hypertrichosis so embarrassing that she refused to take diazoxide and her treatment was changed to propranolol and bethanidine. The hypertrichosis continued to increase in severity for about a year after discontinuing diazoxide, but it then gradually regressed, and almost completely disappeared during the next 12 months.

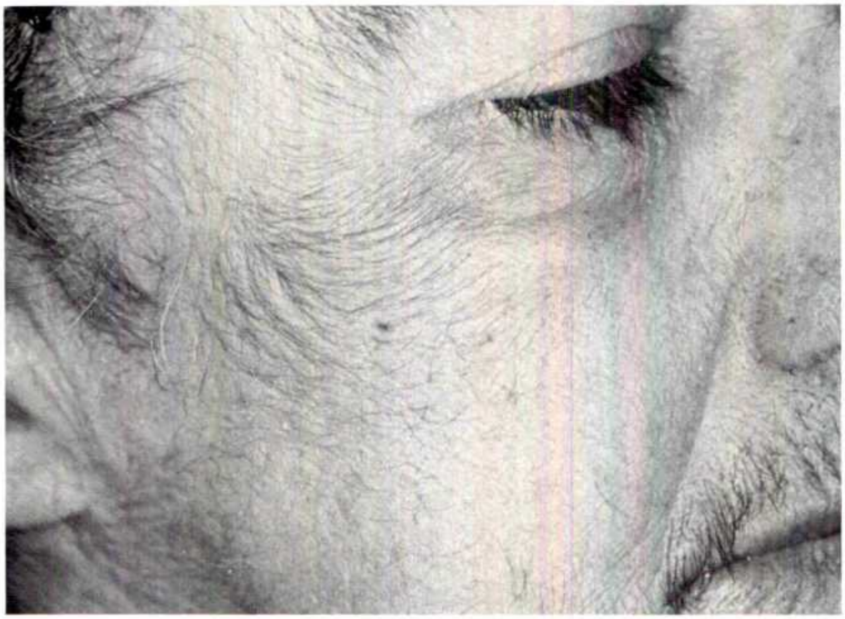

FIGURE 3. Case 2, aged 77 years, showing the hypertrichosis of the face, with widespread growth of pigmented hairs.

DISCUSSION

Hypertrichosis appears to be a frequent side-effect in children treated with diazoxide for long periods. Baker *et al.* (1967) reported that it occurred in all eight of their patients with idiopathic hypoglycaemia of infancy who were treated with diazoxide for between 4 and 18 months. The hair growth was noted as early as 6 weeks after the start of treatment and it affected predominantly the forehead, sideburns, nape of neck, eyebrows and dorsal aspect of trunk and limbs. They described this as vellus hair but the photograph in their report shows clearly that their patient had long blonde hair of the intermediate type rather than the vellus hair seen in normal children. The development of secondary sexual hair was not seen in any of the cases, and they had normal urinary excretion of 17 keto- and hydroxysteroids. A skin biopsy of the affected area in one of their patients showed many hair follicles in anagen, with large amounts of glycogen and a rich vascular network. Two similar cases in children have been briefly reported by Mereau, Kassoff & Goodman (1966), and four infants born to women treated with diazoxide for hypertension during the last trimester of pregnancy developed alopecia (Milner & Chonksey, 1972). This manifested itself at the age of 1 week as abnormal scalp hair which became thin in patches over the skull vault. The alopecia persisted until the infants were last seen (up to the age of 1 year). One of the four affected infants also had hypertrichosis lanuginosa which persisted after birth.

Hypertrichosis due to diazoxide appears to be much rarer in adults than in children. We have found no previous case in the literature and it probably occurs in less than 1% of adults treated with diazoxide (Schering Corp., 1966). Reversibility of diazoxide-induced hypertrichosis has not previously been reported, but this may be because the drug cannot usually be discontinued in children with hypoglycaemia.

The mechanism of the hypertrichosis is unknown. The other side-effects of diazoxide, which are numerous but relatively infrequent, appear to offer no clue. They include transient neutropenia, depression of immunoglobulin levels, an increased serum uric acid level, ketosis, fluid retention and advancement of bone age with epiphyseal maturation. Diazoxide, in common with many other hypotensive drugs, can alter regional blood flow (Rubin *et al.*, 1962) and this may increase cutaneous perfusion. Hyperaemia of skin may increase hair growth, since hypertrichosis occasionally occurs in inflamed skin and over the site of arterio-venous aneurysms (Ressmann & Butterworth, 1952; Allen, 1954; Rook, 1972a). This seems an unlikely explanation, however, since other hypotensive drugs with a similar action do not produce hypertrichosis. A direct pharmacological effect on the hair follicle is another possibility. Diazoxide is an inhibitor of the enzyme phosphodiesterase (Moore, 1968) which breaks down cyclic AMP, and the resulting increase in intracellular cyclic AMP levels might stimulate hair growth.

Other drugs known to cause hypertrichosis without virilization include hexachlorobenzene (Cam & Nigogosyan, 1963), phenytoin (Bray, 1959; Livingstone, Peterson & Boks, 1955), glucocorticoids (Rook, 1972b) and psoralens (Elliott, 1959; Singh & Lal, 1967). The mechanism by which these drugs produce hypertrichosis is also unknown. Hexachlorobenzene is a hepatic toxin which produces porphyria cutanea tarda, but children with diazoxide-induced hypertrichosis have normal phorphyrin excretion (Mereau *et al.*, 1966, Baker *et al.*, 1967). Streptomycin therapy has also been said to produce hypertrichosis (Fono, 1950; Buffoni, 1951), but Holzel (1951) has pointed out that hypertrichosis is seen in children with tuberculosis who have never received streptomycin.

ACKNOWLEDGMENTS

We thank Dr R. P. Dawber, who examined the hair by scanning electron mciroscopy, and Dr L. A. Phillips, of Allen and Hanburys, who provided details of the pharmacology of diazoxide.

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British Journal of Dermatology (1975) **93**, 713.

Acquired pigmentation of skin folds with the histological picture of seborrhoeic wart

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Accepted for publication 10 March 1975

SUMMARY

The case history is presented of a woman with acquired lesions in the axillary, submammary, intermammary, inguinal, genitocrural and perianal skin folds. These lesions were confluent pigmented macules, giving rise to extensive (*en nappe*) reticulate patches. Histology revealed changes typical of seborrhoeic wart.

The name patchy (*en nappe*) seborrhoeic wart is suggested for this condition.

At the Royal Society of Medicine in February 1971, Smith, Dowling & Wilson Jones (1971) presented a case under the heading of 'Acquired axillary and inguinal pigmentation: an epidermal naevoid abnormality not to be confused with acanthosis nigricans'. They referred to two similar cases reported in the literature (Behdjet, 1932; Dowling & Freudenthal, 1938) as acanthosis nigricans unassociated with malignancy.

Jacyk & Miklaszewska (1973), reporting a case of progressive macular pigmentation of the axillae and the groins, thought that the descriptive term 'acquired (or tardive) axillary pigmentation' as proposed by Smith *et al.* seemed the most suitable.

The present paper deals with what would appear to be the fifth case reported in the literature.

CASE HISTORY

A woman, aged 69 years, consulted us for a varicose leg ulcer. She also stated that, some 11 years before, small brown spots began to appear along the submammary folds and that they had slowly spread. A few years later similar lesions appeared in the axillae, groins and genitocrural folds.

Examination revealed reticulate areas of pigmentation in the submammary, intermammary, axillary, inguinal, genitocrural and perianal skin folds (Figs 1 and 2). All the lesions were similar, consisting of brown macules from 1 to 4 mm in diameter coalescing to give rise to reticulate patches. Similar but isolated lesions were found on the back of her neck. There was no desquamation and no

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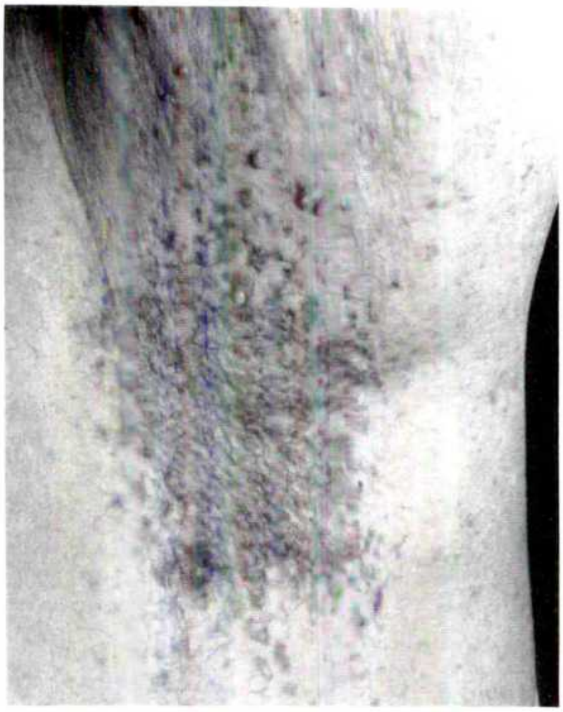

FIGURE 1. Seborrhoeic wart patch: left axilla.

subjective symptoms. Molluscum pendulum lesions, which the patient stated had been present since youth, were noted in both axillae.

Except for the varicose ulcer the rest of the dermatological examination as well as the laboratory findings were non-contributory.

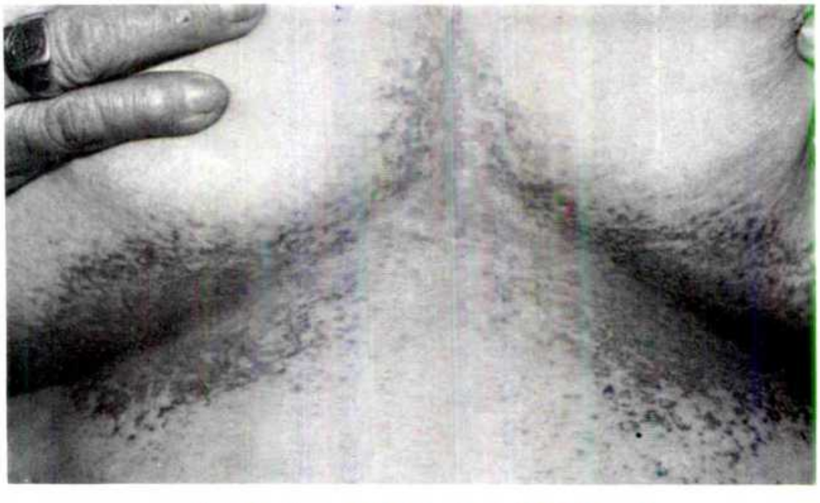

FIGURE 2. Seborrhoeic wart patch: sub- and intermammary areas.

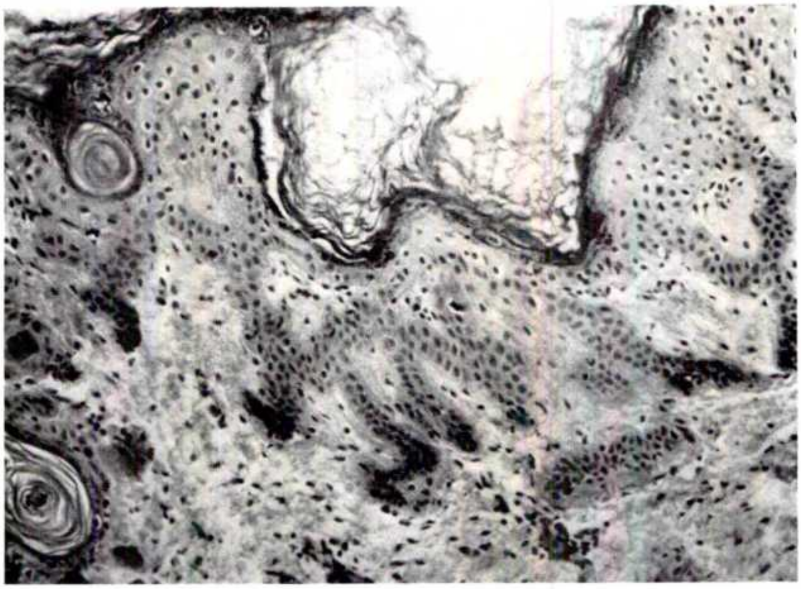

FIGURE 3. Slight epidermal hyperkeratosis. Melanin pigmentation is observed at the base of the rete ridges (H & E stain, × 100).

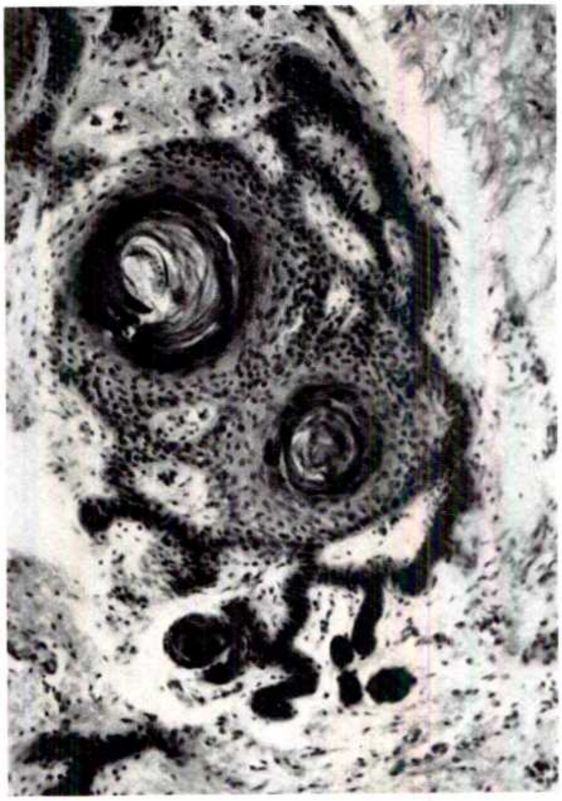

FIGURE 4. In the upper dermis are epithelial proliferations with trichoepithelioma-like characteristics, originating in the external epithelial sheath of the hair follicle (H & E stain, × 100).

Histopathology

Biopsy specimens were obtained from five sites (two from the axillae, one from the intermammary area, one from the submammary area, and one from the groin). They all revealed similar changes. The epidermis showed moderate hyperkeratosis and acanthosis. Hyperkeratosis was also observed in the hair follicles and in some sections it simulated horny inclusion cysts. No abnormalities were apparent in the stratum granulosum. The prickle cell layer showed thinned and elongated interpapillary projections accompanied by increased pigmentation in the basal layer cells. A few perivascular histiocytes, lymphocytes and melanin-containing macrophages were present in the papillary and the reticular layers of the upper corium. No abnormality was evident in the hypodermis or in the sweat coils. The axillary biopsy showed epithelial villi with trichoepithelioma-like characteristics, arising from the outer epithelial sheath of the hair follicle. The histopathologist's report was: adenoid seborrhoeic wart (Figs 3 and 4).

COMMENT

Smith *et al.* commented that in their patient, except for the follicular involvement, the histology 'closely resembled that of a flat adenoidal seborrhoeic wart'.

It seems to us that the time has come to find a specific name for this condition, which is evidently different from what is classically known as acanthosis nigricans and with which it may have been confused in the past. We feel that this name should give an idea of the clinical as well as of the histological characteristics, for instance: patchy (*'en nappe'*) seborrhoeic wart. This avoids all reference to acanthosis nigricans with its different histology, and emphazises the verrucous appearance of the lesion.

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British Journal of Dermatology (1975) **93**, 717.

Brief Communications

Wart immunity, autoantibodies and Australia antigen in sarcoidosis

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Accepted for publication 5 April 1975

Twenty adult patients with clinical, histological and radiographic evidence of sarcoidosis were investigated for the presence of cell-mediated and humoral immunity to wart antigen. Four had a past history of warts but none had clinical evidence of the infection at the time of the investigation. Cell-mediated immunity, as measured by the leukocyte migration inhibition test (Morison, 1974), was absent in all cases. 90% had antibodies to wart antigen as demonstrated by two methods, haemagglutination inhibition and counter-current immuno-electrophoresis (Morison, 1975). The antibody was IgG in all cases. This compares with a 37% incidence of wart antibodies in forty-one control subjects. The questions raised are: did these patients have poor memories and not remember their past warts? Alternatively, is the increased incidence of antibodies to wart antigen an indication of subclinical infection with wart virus? There have been recent reports of an increased incidence and titre of antibodies to a number of viruses in patients with sarcoidosis (Wahren *et al.*, 1971; Byrne *et al.*, 1973). These viruses include herpes simplex, cytomegalovirus, Epstein-Barr, rubella and parainfluenza. It is known that in sarcoidosis, probably to compensate for defective cell-mediated immunity, patients may have an over-reactive humoral immune system and they respond with excessive antibody production to immunization (Sands *et al.*, 1955). It is therefore possible that subclinical viral infections are common and that the small antigenic stimulus is sufficient to trigger off a sustained antibody response.

The patients' sera were also examined and found negative for: Australia antigen even on radioimmunoassay; antinuclear factor; antibodies to gastric parietal cells, mitochondria, smooth muscle and biliary canaliculi using a standard indirect immunofluorescent technique. These results agree with the findings of other workers (Sharma *et al.*, 1970; Tolk *et al.*, 1972).

ACKNOWLEDGMENTS

Dr June Almeida kindly performed the Australia antigen studies and Mrs P. Hilson the examination for auto-antibodies.

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Pigmentation of the palms and scalp probably due to proprietary hair tonics, containing various phenols and phenolic derivatives

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Accepted for publication 25 April 1975

A 37-year-old man used a proprietary hair care and hair tonic, which he applied quite vigorously to the scalp for 2 years. The lotions were poured into the palms and then rubbed into the scalp.

One of the preparations contained: trichlorohydroxy diphenyl ether 0·2%, phenol 0·2%, *o*-cresol 0·15%, thymol 0·5%; and the other 0·5% N-pantoylethoxypropylamine.

About 3 weeks before he was seen he noted that the skin of the centre of his palms was becoming light yellow. This spread and the colour deepened. The whole of the palms, the sides of the fingers and the finger webs became dark brown. The skin of the temples and hair margins and to some extent the scalp showed a similar tint. There was no evidence or symptoms of any associated dermatitis. When he stopped using the applications the pigmentation of the palms and temples disappeared in 2 weeks without exfoliation.

Dr Lawrence Gent of the Biochemistry Department, Guy's Hospital, states 'The various phenols, quinoline, and the diphenyls are all capable of being photoactivated, and of being condensed to form polynuclear quinonoid compounds which are often coloured'.

Erythema nodosum from *Yersinia enterocolitica*

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Accepted for publication 29 May 1975

There have been a number of reports from Scandinavia and France incriminating *Yersinia enterocolitica* (YE) as a cause of erythema nodosum (EN) (Mollaret & Destombes, 1964; Mygind & Thulin, 1970; Hannuksela, 1971; Leino & Kallionaki, 1974). Hannuksela reported that one in ten cases of EN in Finland was caused by YE.

We are unaware of any such cases having been reported from the U.K., although there have been reports of EN due to *Yersinia pseudotuberculosis* (Turner & Wilkinson, 1969; Wilkinson, Turner & Mair, 1969).

CASE REPORT

Whilst on holiday in Ibiza, a 24-year-old female developed diarrhoea, associated with colicky abdominal pain. This acute illness settled on her return to England 2 days later. After a further 16 days, she developed red, painful nodules on both shins and presented at the Casualty Department 3 days after their onset.

On examination, she had classical EN affecting the anterior aspects of both lower legs. There was no associated pyrexia and clinical examination revealed no other abnormal findings.

The following investigations were normal or negative: Haemoglobin; A.S.O. titre; urine culture; throat swab; stool microscopy; chest X-ray and E.C.G.

E.S.R. was 60 mm in 1st h (Westergren); white cell count 10,600 per mm^3, with 81% polymorphs; Tuberculin test weakly positive; culture of faeces revealed a mixed growth of normal flora only. YE type 3 antibodies were detected in the serum, to a titre of 1:640. Ten days later, the titre had fallen to 1:160, these results being consistent with active infection.

On receipt of the initial serology, she was given a 10-day course of tetracycline; the EN cleared rapidly.

With many people now spending brief periods abroad, we feel that YE infection should be considered routinely as a cause of both EN and 'traveller's diarrhoea' in the U.K.

ACKNOWLEDGMENT

We would like to thank Dr N.S. Mair for performing the serology

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British Journal of Dermatology (1975) **93**, 721.

Comment

β-CAROTENE AND THE PHOTODERMATOSES

Classification of the photodermatoses is unsatisfactory because the aetiology of so many is obscure but Ramsay (1975) has suggested four groups: (i) idiopathic; (ii) drug or chemical induced; (iii) due to metabolic disease; (iv) sunlight precipitated or aggravated.

Group (i) includes polymorphic light eruption, solar urticaria, actinic reticuloid and photosensitive eczema; group (iii) erythropoietic protoporphyria (EPP).

EPP was first clearly described by Magnus *et al.* (1961); it is an inborn error of porphyrin metabolism characterized by an excess of red cell and perhaps faecal protoporphyrin but with normal urinary porphyrin excretion. The cardinal symptom is photosensitivity, often beginning in childhood, frequently manifesting as a severe burning sensation after only brief exposure to sunlight. The responsible wavelength is around 400 nm.

It has long been known that carotenoid pigments could protect photosynthetic bacteria from lethal photosensitivity (Sistrom, Griffiths & Stanier, 1956) but it was not until 1969 that the carrot pigment, β-carotene, was shown to be successful in the treatment of three patients with EPP (Mathews-Roth *et al.*, 1970 a, b).

Reports of its value in the management of EPP and some other photodermatoses have appeared regularly since then, and last year a small group of physicians met in London under the chairmanship of Dr Ian Sneddon to discuss their results and experiences since β-carotene became available in the U.K. in 1971.

Among the many reports which have appeared (Fitzpatrick *et al.*, 1971; Mathews-Roth *et al.*, 1972; Lewis, 1972; Baart de la Faille *et al.*, 1972; Ippen, 1972; Moynahan & Leppard, 1973; Krook & Haeger-Aronson, 1974; Mathews-Roth *et al.*, 1974) controlled trials have been conspicuously absent. The conventional 'double-blind' technique is impossible because of the unavoidable skin staining which results from the hypercarotenaemia during treatment with β-carotene. In some individuals carotenoderma is quite striking but the majority find it cosmetically acceptable. Replacing some of the β-carotene with another synthetic carotenoid pigment, canthaxanthin, modifies the carotenoderma in such a way that the appearance is much more like natural sun-tan. Inevitably, both in EPP and in other photodermatoses the effectiveness of treatment must rest on subjective assessment, together with the physician's judgment, hopefully reinforced by measurement of relatively objective parameters such as the determination of changes in reactivity of the skin to artificial radiation. Nevertheless, because of the wide variability in this reactivity not only between subjects but also according to time of day, skin temperature, humidity and so on, assessment of effect by such artificial means is of limited value. Consequently, results of 'trials' with β-carotene must be assessed principally on single case reports rather than on formal trial groups. Donaldson (Stoke-on-Trent) has treated five EPP patients (4 female, 1 male) with oral doses of β-carotene (Roche) of between 150–200 mg daily, in divided doses, over a period of 3½ years. Three were improved, one reported no change and one was lost to follow-up. Wide fluctuations in RBC porphyrin were noted whether patients were on treatment or not. However, two of the three improved patients were shown to have a 10-fold increase in tolerance to Xenon Lamp exposure (400–600 nm) while on β-carotene. Serum carotene levels ranged from 100–520 μg/100 ml; one patient clearly showed a dose-related response. Although Fitzpatrick *et al.* (1971) have suggested that plasma levels of at least 400 μg/100 ml are necessary to achieve any therapeutic effect, Donaldson found improvement in some of his patients to be associated with low serum concentrations.

Others at the meeting reported similar or better results in EPP, both in children and adults. In all, some forty-four patients have been treated with β-carotene in the U.K. in the last 3 or 4 years and forty-two have reported benefit ranging from merely 'improved' to 'transformed life'. Two patients with congenital porphyria (Gunther) are also reported to have benefited but one patient with porphyria cutanea tarda has not responded.

The situation in other light sensitive dermatoses is not so clear. Between 40 and 60% of patients with photosensitive eczema, polymorphic light eruptions or solar urticaria appear to respond to a varying extent. In the last 2 years Holti and his colleagues (Newcastle-upon-Tyne) have treated twenty-one patients in the 'idiopathic' group and, in contrast to the experience of Kobza, Ramsay & Magnus (1973), have had encouraging results. Only one of eight and two of nine patients with solar urticaria and light provoked eczema respectively, completely failed to respond. The others showed varying degrees of benefit from this treatment but none was rendered symptom-free. This, however, also applies to patients with erythropoietic protoporphyria.

An objective test of therapeutic effectiveness is badly needed. One of Holti's patients with severe solar urticaria, a bell-ringer, volunteered that his change-ringing sessions left him with intact palms while on treatment, whereas previously he could not handle the ropes for more than 15 min without blistering. Could β-carotene have an epidermal thickening effect?

To try to answer that question, and at the suggestion of Comaish, suction blister times (Comaish & McVittie, 1973) were measured in the bell-ringer and fifteen other patients before, during and after treatment. Significantly increased times were demonstrated during treatment compared with those before and 6 weeks after treatment. In three patients whose blister times did not alter significantly β-carotene had conferred no benefit. On the other hand, one patient who claimed a 50% improvement on β-carotene had no change in blister times. The suction blister test is not easily standardized and results are dependent on other variables such as site, skin temperature, humidity and so on. Consequently it is difficult to know how much reliance can be placed on these results; more data are obviously needed.

β-carotene is, of course, a provitamin A; indeed it is the most important of the precursors in the animal world with a *theoretical* potential for conversion to retinol in gut mucosa in the ratio 1:2. However, man is a very inefficient converter and it is doubtful if more than 10% of dietary β-carotene is converted to the vitamin in normal circumstances. Plasma retinol levels may rise in only a small number of patients during treatment with β-carotene but remain within the normal range. Daily intakes of 100 mg β-carotene would normally raise plasma carotene levels to between 600 and 2000 μg/100 ml in 3 months; plasma retinol levels would average about 250 iu/100 ml (≡75 μg/retinol). There is therefore no real risk of inducing hypervitaminosis A in patients during oral β-carotene treatment although, because of biological variability in conversion rates, it is probably wise to monitor vitamin A levels during treatment (Nieman & Obbink, 1954; Bagdon, Zbinden & Studer, 1960; Lewis, 1972).

Canthaxanthin, mentioned earlier, is a synthetic carotenoid with *no* provitamin A activity. On its own in oral doses of the same order as β-carotene, it produces an unpleasant and unacceptable deep salmon-pink skin colour. With added β-carotene, however, the effect is modified and resembles natural tanning. A combination of 10 mg β-carotene and 15 mg canthaxanthin appears to be as effective in EPP as 25 mg of pure β-carotene but therapeutic trials with the mixture have not been as extensive as with the latter alone.

There are many unanswered questions in the β-carotene story. Individual case reports from U.S.A., U.K. and Europe provide circumstantial evidence that 90% of patients with EPP benefit, so that many dermatologists are sufficiently convinced of its value and see no need for more scientific assessment by controlled clinical trial. In EPP no other treatment has so far been found as effective;

nevertheless, Ramsay and others feel that a controlled study should at least be attempted, although not even a 'single blind' trial is possible.

If β-carotene has an effect in these conditions how is it mediated? Because it apparently accumulates in skin is it acting simply as a sunscreen? In fact, monochromator studies in protected patients give no support to that idea; further, there is no evidence that β-carotene even reaches the epidermis and indeed topical application is singularly unsuccessful.

A more elegant hypothesis is based on the fact that β-carotene is, *in vitro*, an effective singlet excited oxygen quencher. There is some evidence that photosensitivity in EPP and indeed in other types of porphyria is due to the presence of an excited state of oxygen. There is some experimental *in vitro* work to support this hypothesis (Davies *et al.*, 1975) but the lack of a suitable animal model hampers further research in this field. A porphyrin-like photosensitivity occurs in sheep in some areas but these animals convert their ingested β-carotene *in toto* to vitamin A.

Gardiner (ICI, Pharmaceuticals Division, Macclesfield) has carried out a controlled trial of β-carotene in thirteen healthy volunteers rendered photosensitive by contact with an anthraquinone-based dye, Disperse Blue 35 (Gardiner, 1974). Photo-contact dermatitis in this situation is to visible light (500–650 nm) but in other respects the model parallels the postulated system for porphyria. In that trial, however, 175 mg β-carotene daily was ineffective as judged by patch and photopatch testing in spite of an average 10-fold increase in plasma carotene levels. However, it was only given for 3 weeks and this, together with an insufficient range of exposure to light, may not have been sufficient for any demonstrable benefit. The trial was carried out in 1974 in ICI Organics Division.

Other *in vitro* studies have purported to demonstrate an inhibitory effect of β-carotene on protoporphyrin-induced photohaemolysis which cannot be explained as a pure 'filter effect' (Swanbeck & Wennerstein, 1973) but their results are open to criticism and the work has not so far been repeated.

Those dermatologists with patients who have shown a response will continue to treat them with β-carotene or β-carotene and canthaxanthin combined simply because these compounds afford relief and make management of otherwise intractable conditions that much easier. The academics are not so happy. As Ramsay pleaded at the meeting '. . . we must be careful about it (using β-carotene), otherwise we will be feeding this drug to our patients for many years and, unless we obtain positive evidence of benefit now, we are not going to know in 10 years' time if we have been right or not'.

I would like to thank Dr Colin Ramsay for helpful suggestions in the preparation of this article and Drs Donaldson, Gardiner and Holti for allowing me to report their results.

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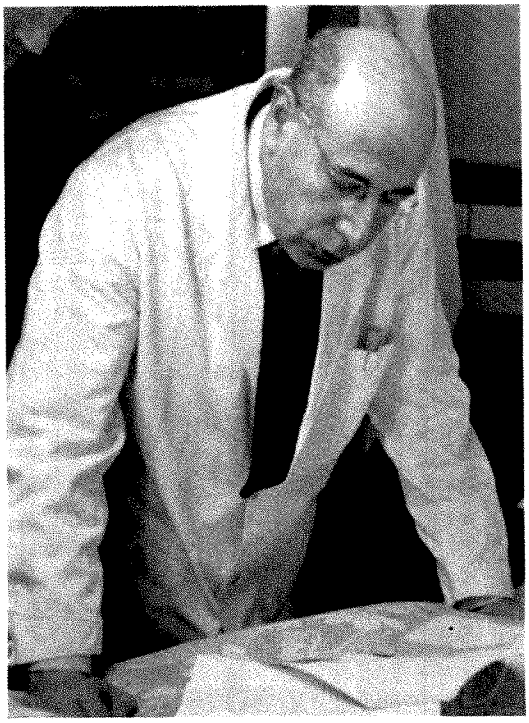
British Journal of Dermatology (1975) **93**, 725.

Obituary

DR W.N.GOLDSMITH

William Noel Goldsmith was born in Kensington on 26 December 1893 and died at the age of 81 on 4 April 1975. His parents were Bertha Feist and Ernst Goldschmidt, one of the founders of the London Metal Exchange. He had three sisters, and a younger brother. An uncle, Viktor Goldschmidt, was Professor of Mineralogy in the University of Heidelberg, and a cousin, Stefan Meyer, was Head of the Radium Institute in Vienna. His childhood was happy—both at home, a spacious house decorated in a comfortably Edwardian style, and at Rugby where he was a pupil from 1907 to 1912. He studied at Pembroke College, Cambridge, until his entry to University College Hospital Medical School in 1915, where his only recorded distinction was a prize in anaesthetics. After qualification in 1918 he served for two years in the Royal Army Medical Corps.



Chronologically, his diplomas and degrees were as follows: B.A.Cantab., 1915 (M.A. 1919); M.R.C.S. Eng., L.R.C.P.Lond., 1918 (M.R.C.P. 1922, F.R.C.P. 1937); M.B., B.Chir.Cantab., 1921 (M.D. 1927). The subject of his Thesis was 'The Staphylococci in Acne Vulgaris'.

His military experience and the advances inspired by the work of Sir Robert Jones awakened in him an interest in orthopaedics but he soon turned to dermatology as his life's work. Precisely why he chose this subject is unknown. He became a Clinical Assistant to A.M.H.Gray in the Department of Dermatology at U.C.H. in 1922 and two years later was awarded the Radcliffe Crocker Travelling Scholarship. This enabled him to spend a year abroad, notably in Vienna, under Otto Kren, and for most of the time in Breslau under Josef Jadassohn. He joined the Consultant Staff at U.C.H. as Assistant Physician to the Department of Dermatology in 1932, succeeding Gray as Physician-in-charge in 1946. He also served on the Consultant Staff of St John's Hospital for Diseases of the Skin from 1928 to 1939 and at various times worked in a number of general and special hospitals—the Kensington, Fulham and Chelsea General Hospital (his first appointment); the Prince of Wales', Tottenham; St John's, Lewisham; the

West Herts, Hemel Hempstead; the West End Hospital for Nervous Diseases, and after his retirement from U.C.H., the Italian Hospital. Throughout his professional life he conducted private practice.

He became a Member of the British Association of Dermatology in 1926 and was made an Honorary Member in 1963. He was Assistant Editor of the *British Journal of Dermatology* under A.C.Roxburgh in 1938 following him as Editor from 1939 until 1948. He was President of the Association in 1958. He was a Fellow of the Royal Society of Medicine, President of the Dermatological Section in 1949–50 and our Library representative for many years, a Member of the St John's Hospital Dermatological Society and its President in 1936–38, and a Member and Vice-Chairman of the Medical and Dental Society for the Study of Hypnosis. Many foreign societies honoured him: he was a corresponding Member of the Dermatological Societies of France, Hungary and Sweden and an Honorary Member of the Australian, Berlin, Danish and German Dermatological Societies, and of the American Dermatological Association.

Goldsmith's first paper was published in this *Journal* in 1924. It described a new mycological medium to replace Sabouraud's, whose ingredients were no longer available. In 1925 he published two papers in German in the *Archiv für Dermatologie und Syphilis*, then edited by Jadassohn and Pick. These were concerned with work carried out in the Breslau Clinic, the first *Arbeit* a clinical study of circinate and verrucous forms of sarcoid and lupus vulgaris, the second a laboratory study of the staphylococci of acne. (Bibliographers should note that these first three papers were published under the name Goldschmidt.) During the next 30 years there appeared a succession of papers, about thirty-five in all, mainly in this *Journal* and concerned with a wide variety of topics, notably allergy, the reticuloses, irradiation and other forms of treatment, including ointment bases.

His major literary activity lay elsewhere. Gray had been asked to write an account of progress in dermatology and delegated the task to his junior colleague. Years of labour culminated in the publication of the first edition of *Recent Advances in Dermatology* in 1936: it was an account of developments during the previous 20 years. The material was marshalled in logical sequence and the reader adjured to 'plough bravely through the chapters in proper order (and then perhaps doing it again)'. Those with the fortitude to do so were amply rewarded and the work was immediately successful and remains greatly admired. A naïve reviewer suggested that it was so good that publisher and author should consider bringing out a new edition every few years (eighteen were in fact to pass before the appearance of the second edition, prepared with the help of F.F.Hellier). Meanwhile, Goldsmith had shouldered the burden of editing this journal during the dark and difficult years of the war and its aftermath.

His lifelong interest in radiotherapy made him an invaluable member of the Panel on External Radiotherapeutic Procedures of the Committee on Radiological Hazards to Patients (the 'Adrian Committee').

Another time-consuming activity was his participation in the Joint Committee of the Royal College of Physicians on Nomenclature of Disease, and the preparation with R.M.B.Mackenna, of the Section on Disease of the Skin, Mucous Membranes, Hair and Nails. The skill, scholarship and devotion which he brought to these tasks are legendary.

In his career, the two main influences appear to have been A.M.H.Gray with his diagnostic flair and infectious enthusiasm, and J.Jadassohn, the scientific genius of post-war dermatology. Gray was his 'unfailing counsellor, friend and supporter', to whom he owed almost everything professionally. Clashes of opinion may be inferred from Goldsmith's comment that Gray could 'show impatience with those who held other views, but differences were amicably composed'. Indeed it is difficult to imagine two brilliant men with more dissimilar personalities. Goldsmith shared neither Gray's appetite for committees nor his pleasure in the exercise of influence. He was wearied by medical politics and the power games of teaching hospital factions—but he did not hold himself aloof. Jadassohn he remembered 'with reverence and affection'. He clearly admired his singlemindedness and commented, with obvious approval, that he was 'shy and the antithesis of a showman'. In the Breslau Clinic he met Frei (who supervised his work on staphylococci), Jessner—a disciplinarian, Biberstein and Freudenthal, the latter particularly befriending him (the roles were to be reversed when Freudenthal, fleeing from Nazi persecution, came to England and was invited to U.C.H. by Gray).

Goldsmith was clinically meticulous. He preferred to examine the patient's skin before taking a detailed history and was insistent upon morphological detail. All the evidence was subjected to close analysis before a diagnosis was made, and the choice of treatment received the same care. He was critical and analytical rather than highly original: his utterly logical approach precluded the speculative leap in the dark, the inspired guess. His literary style was lucid and often surprisingly vivacious. A single example is a sentence from a paper on allergy: 'Now antibodies are elusive elves, and it will not do if in every instance, before calling a manifestation allergic, we have to ask ourselves—do you believe in fairies ?'

Some misjudged Goldsmith's solemn expression; there was indeed about him a lot of the Roman *gravitas* but he was never pompous. Although a man of exceptional erudition, he was basically humble. He enjoyed life and found people funnier than they often suspected. He was intensely musical, although he confessed to being an indifferent performer. He subscribed to *The Gramophone* from its first publication and had a collection of records which was subjected to the same scrutiny and revision as all other aspects of his activities. He followed the fortunes of the English ballet companies from their infancy and had the reputation of being an excellent ballroom dancer himself—he confessed that he had found the Charleston 'intoxicating'. He was a patient and skilled photographer but so modest that few saw the results of his labours. An entertaining host, he amazed with witty conversation and anecdote those accustomed to the long, thoughtful silences of his professional dialogues. Seemingly a confirmed bachelor, he married after his retirement. His later years were burdened with multiple and painful illnesses through which he was sustained by the devotion of his beloved Irene. To her and Dorothy, his surviving sister, we offer our condolences.

With the passing of this great and gentle man we lose one of the outstanding and memorable figures of British, indeed World, dermatology. His influence remains in the work of those who had the honour to study under his guidance and in the distinction of our British dermatological literature including this journal which he saved from oblivion during the war and raised to a level of enduring excellence.

P.J.HARE

Dermatology has lost in William Goldsmith an outstanding figure, and others will record and assess his contributions to our discipline, but to me his passing means the loss of a very dear friend. He had a real genius for friendship, and in 45 years of our intimacy I cannot recall him ever saying anything hard about any person. He knew everybody who was anybody in world dermatology, and was extremely helpful to younger colleagues through his ability to tell them where to find what had already been done in a field which had attracted their attention. Latterly his bodily infirmities had cut him off from some of his favourite activities, but he kept his personality intact to the end.

J.FERGUSON-SMITH

DR FRED GLYN-HUGHES

Dr Fred Glyn-Hughes' invalid father had a large family and although young Fred had won a scholarship he was forced to leave school before matriculating. His passion was chemistry as one might suspect of someone from Runcorn, and he started life as a lab boy at Liverpool University. The Professor encouraged him to take London external matric: and this was to be his key to a medical career. He was delayed both by the First World War and by an attack of phthisis, fortunately not severe. Schoolteachers were scarce at the time, and he taught chemistry at Liverpool College. Quite a number of our colleagues of today met him either at the College or in the laboratory and have admired him for his subsequent achievements. His medical fees were paid with the help of a substantial University loan, and the little extras came from coaching others in chemistry.

There followed a period of 7 years in General Practice, but his spare time was spent in the Dermatological clinics. Liverpool between the wars was on the verge of poverty. Hospital beds were of necessity in extremely generous supply, as were the patients. Fred Glyn-Hughes jumped at an opportunity to enter Dermatology through the Corporation hospitals. His department had previously been started as a small isolation unit for scabies and impetigo. It rapidly grew when he was confronted by hordes of obese multiparous women with even greater hordes of leg ulcers, by droves of cases of skin sepsis of every sort, by hundreds of patients with lupus vulgaris and the like. His M.D. was gained after research on lupus vulgaris but his main achievement was in organizing a dermatological service in every major Corporation hospital in the city. One of his secrets was to train nurses in the whole-time management of skin diseases and not to be afraid of them. They spent their career with him and gave him their all. Another was the art of leadership; to bring his patients in, as he said, out of the wilderness. It spread his fame far beyond its original confines. One of his proudest moments at this time was to pay back to the University every penny he owed, much to their astonishment.

Wild horses wouldn't keep him away from the meetings of the North of England Dermatological Society, of which he became an honoured President, and the B.A.D. was to him unalloyed joy. His personality was in many ways unique. Bursts of tempestuous energy would be followed by drained exhaustion. His early phthisis had given him a complete loss of confidence in what can only be described as the rudest of rude health. He learned the Welsh of his parents and loved to practise it on the unsuspecting. No Welshman could ever do wrong. He loved everything Welsh, from football to feathered friends and became President of the North Wales Ornithological Society.

His wife, also a doctor, was a lifelong student. He vied with her in avidly reading Medicine and the English Classics. He was never at a loss for a turn of phrase. One only need be a listener in his presence, but what stimulating listening it could be.

He died on 16 October 1974 in Colwyn Bay, aged 77, still remembered by the Liverpudlians he had left 13 thirteen years before.

C.McGibbon

British Journal of Dermatology (1975) **93**, 729.

Society Proceedings

Irish Dermatological Society

3 May 1975, St. Vincent's Hospital, Dublin
Chairman: DR DONAL BUCKLEY

Necrobiosis lipoidica diabeticorum involving scalp and face. DR J.P.MACKEY

A woman aged 67 years.

History. The mother and two sisters of this patient had diabetes mellitus, and the first hint that she, too, might be diabetic was at the age of 28 when she produced a 5·27 kg baby.

When she was 38 years old, she had her last confinement which was complicated by puerperal sepsis and possibly by a deep vein thrombosis in her left leg. A rash appeared on that leg 2 years later. The eruption subsequently involved other sites: her scalp after a further 16 years and her face and arms 8 years after that; by then she was 64 years old. She became overtly diabetic 1 year ago.

Examination. On her scalp there was a large atrophic plaque through which the subcutaneous veins were readily visible (Fig. 1). The edge of the lesion was indurated and it extended onto her forehead. There was a small lesion below her left eye and a few more on her left arm. Several subcutaneous nodules were found on her left wrist, and the skin of her legs was atrophic and pigmented (Fig. 2).

Investigations. Blood: The Hb was 13·5 g%; the WBC was 4900/cmm, and the differential count showed neutrophils 62%, lymphocytes 31%, eosinophils 2%, and monocytes 5%. The ESR was 51 mm/h.

Biochemical: The serum cholesterol was 192 mg/dl (150–250), triglycerides 146 mg/dl (10–190), and calcium 9·3 mg/dl (9–10·6).

The blood glucose was 130 mg/dl (60–100) fasting, and 265 mg/dl postprandially.

Bacteriological: A vaginal swab grew out Candida.

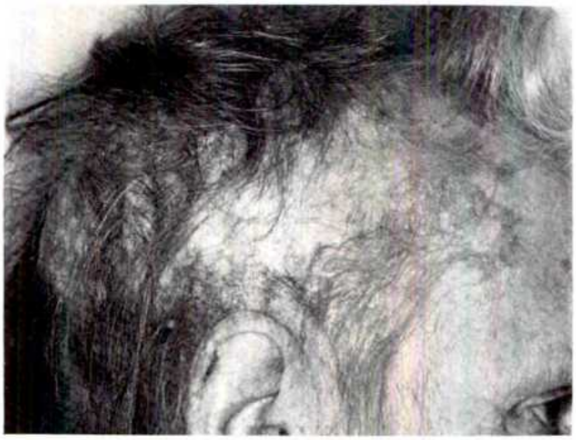


FIGURE 1. Large atrophic plaque on the scalp through which the subcutaneous veins are readily visible.

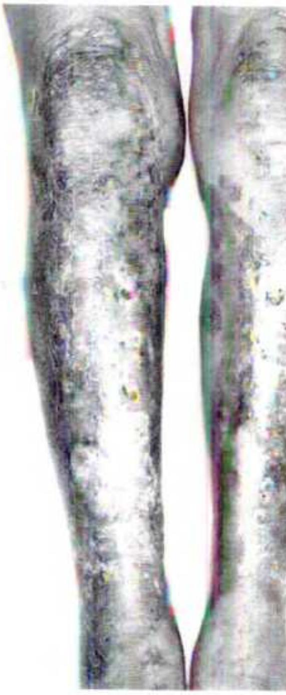


FIGURE 2. Skin of the legs showing atrophy and pigmentation.

Radiological: An X-ray of her left wrist showed no evidence of sarcoidosis in bone; a chest X-ray was normal.

Histology of a biopsy taken from the left leg to include skin and a deep wedge of subcutaneous tissue, was reported as follows:

'The features shown in this section are essentially similar to those seen in previous skin biopsies (taken from the forehead), but the dermis is rather less crowded by granulomas. There is a small number of reasonably convincing areas of collagen necrosis, in which granuloma formation is only commencing. In the central zones of the better formed granulomas, there are numerous vacuoles suggesting the presence of substantial quantities of fat, even though these are situated in the dermal collagen and not in the subcutaneous fatty tissue. A number of small arterioles show some hyaline thickening of their wall and swelling of their endothelial cells. I do feel that the findings strongly favour necrobiosis lipoidica.' (Professor M.Hickey.)

Comment. Necrobiosis lipoidica is comparatively rare, being present in only 3 per 1000 diabetics. Only two of the twenty-nine cases of necrobiosis lipoidica presenting on the scalp, reported by Wilson Jones (1971), had manifest diabetes; in one of these two patients the skin disease had antedated the diabetes by 14 years, although she had produced heavy babies.

The present case is of interest, as overt diabetes developed 39 years after the first suspicion of a diabetic tendency, and 27 years after the appearance of necrobiosis lipoidica diabeticorum.

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British Journal of Dermatology (1975) **93**, 731.

Correspondence

HIGH DOSES OF ASCORBIC ACID

SIR, High doses of ascorbic acid (3 g daily) may help some patients with indolent ulcers including thalassaemia leg ulcers (Afifi *et al.*, 1975); also, antioxidants such as ascorbic acid, may prevent haemolysis in thalassaemia major (Giardini, Castro & Maggioni, 1974). Afifi *et al.* (1975) state that high doses of ascorbic acid are non-toxic. However, 2 g or more daily of ascorbic acid may cause infertility in women after 6–17 months (Briggs, 1973a, 1973b) and affect calcium and phosphate metabolism in animals (Brown, 1973). In addition those patients who have a high capacity to convert ascorbic acid to oxalate may develop renal stones (Briggs, Garcia-Webb & Davies, 1973). Although Briggs (1973c) reported a patient having a renal stone after taking 2 g of ascorbic acid daily for 2 weeks, the precise time relationship between ascorbic acid therapy and oxalic acid stones is unknown. Therefore high doses of ascorbic acid may be toxic in some patients.

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British Journal of Dermatology (1975) **93**, 732.

News and Notices

THE ROYAL COLLEGES OF PHYSICIANS

The following Dermatologists have recently been elected to the Fellowship of the Royal Colleges of Physicians:

FRCP (*Edinburgh*)	Lt Col. T.C.Hindson.
FRCP (*Glasgow*)	Dr W.N.Morley.
FRCP (*Ireland*)	Dr W.H.McDaniel (1974).
	Dr K.Scott (1974).
	Dr D.B.Buckley (1975).

APPOINTMENTS

Dr Dorothy Thompson has been appointed Consultant Dermatologist to the West Sussex Region. She graduated M.B., Ch.B. in 1964, from Glasgow University and became M.R.C.P. (U.K.) in 1970.

Her early training in medicine was in Glasgow and later Sheffield and Nottingham; her training in Dermatology has been in Nottingham and Aberdeen and recently University College Hospital, London. Her main research interests have been concerned with the mode of action of dapsone in immune complex disease.

Index

SUBJECTS

- Acid, ascorbic; high doses of (letter), 731
- Acne and eczema; familial study (summary), Suppl. 11, 10
  - lipase, hyaluronidase and protease production from bacteria isolated from patients with (summary), Suppl. 11, 26
- Acromegaly; epidermal structure and metabolic activity in (summary), Suppl. 11, 29
- ACTH in the treatment of lichen sclerosus et atrophicus, 215
- Adenosine 3′,5′-monophosphate, cyclic (cyclic AMP); epidermal levels in psoriasis, 253
- Adenyl cyclase in the epidermis; effects of catecholamine and related compounds on, 29
  - (normal and psoriatic); ultrastructural localization and differentiation of, 495
- Adhesion, dermo-epidermal; effect of on epidermal structure in the mouse, 417
- Alcohol injections, multiple intradermal; in the treatment of pruritus vulvae, 201
- Alopecia areata (letter), 605
  - cicatricial, with follicular keratosis (Graham-Little syndrome) (case report), Suppl, 11, 53
- Amino acids and lymphocytes; nickel binding to, 557
- Amyloidosis, urticaria and deafness. Muckle Wells syndrome (case report), Suppl. 11, 46
- Angiitis, necrotizing; associated with mycosis fungoides (case report), Suppl. 11, 62
- Angioma serpiginosum; familial incidence, 701
- Animal model for screening topical and systemic drugs for potential use in the treatment of psoriasis (summary), Suppl. 11, 27
- Antibiosis, natural, among skin bacteria as primary defence against infection, 487
- Aspirin and food additives: reactions to in patients with chronic urticaria, including the physical urticarias, 135
- ATP-hydrolytic activity, membrane-bound: of normal and psoriatic epidermis, 495
  - of psoriatic epidermis, 469
- Australia antigen, wart immunity and autoantibodies in sarcoidosis, 717
- Autoradiography: studies in nickel hypersensitivity, 557
  - study of epidermal transit times, 649

- Bacteria: Gram negative, on skin; physiological and environmental control of, 191
  - isolated from acne patients; lipase, hyaluronidase and protease production from (summary), Suppl. 11, 26
  - skin bacteria; natural antibiosis among, as primary defence against infection, 487
  - *Yersinia enterocolitica*, causing erythema nodosum, 719
- Baldness, male pattern; associated with rosacea, 63
- Benzalkonium chloride; patch test reactions to, in twin pairs, 145
- Benzoates and tartrazine causing anaphylactoid purpura (case report), Suppl. 11, 61
- Biochemistry:
  - absorption and biotransformation of topically applied 8-(methylthio) cyclic AMP, in dogs and humans, 379
  - cyclic AMP levels in psoriatic epidermis, 253
  - effects of catecholamine and related compounds on the adenyl cyclase system in the epidermis, 29
  - erythrocyte glucose-6-phosphate dehydrogenase in lichen planus, 353; (letter), 354
  - inhibition of lysosomal enzymes by dapsone, 471
  - lysosomal hydrolases of the epidermis. 1. Glycosidases, 1; 2. Ester hydrolases, 391; 3. Peptide hydrolases, 509
  - membrane-bound ATP-hydrolytic activity of psoriatic epidermis, 469
  - studies on the energy metabolism in lichen planus, 667
  - thin layer chromatography and counter-current analysis in porphyrias, 277
    - use of in separation of free porphyrins and porphyrin methyl esters, 291
- Blood flow of forearm skin; fluctuations in (summary), Suppl. 11, 25
- Bone marrow transplantation; followed by lichen planus-like eruption, 675
- Book reviews:
  - *Annual Review of Allergy 1973*, ed. C.A.Frazier, 608
  - *Atlas de Mycologie Médicale*, J.Delacrétaz, D. Grigoriu and G.Ducal, 356
  - *Atopic Dermatitis.* Major Problems in Dermatology, Vol. 3, G.Rajka, 474
  - *Clinical Aspects of Immunology*, 3rd edition, ed. P.G.H.Gell, R.R.A.Coombs and P.J.Lachmann, 607
  - *Dermatology—Diagnosis and Treatment of Cutaneous Disorders*, 3rd edition, W.D.Stewart, J.L. Danto and S.Maddin, 475
  - *Dermatophytes in Human Skin, Hair and Nails*, J.T.Sinski, 113
  - *Disorders of the Autonomic Nervous System*, R.H. Johnson and J.M.K.Spalding, 236
  - *Haut-veränderungen bei inneren Krankheiten*, 2nd edition, I.Pastinszky and I.Racz, 475
  - *Immunological Aspects of Allergy and Allergic Disease.* Vol. 1. *Basic Concepts in Experimental Immunology.*

Book reviews (*cont.*)
    Vol. 2. *Methods in Experimental Immunology*, ed. E.Rajka and S.Korossy, 358
  Introduction to Chemical Nomenclature, 4th edition, R.S.Cahn, 235
  Manual of Contact Dermatitis, S.Fregert, 235
  Nail Diseases in Internal Medicine, P.de Nicola, M.Morsiani and G.Zavagli, 608
  Organpathologie, Vol. 3, ed. W.Doerr, 237
  Proceedings of the XIVth Congress of the Association of French-speaking Dermatologists and Syphilologists, Geneva, 1973. Vol. I. *Toxicodermies*. Vol. II. *Vascularites*, 609
  Recent Advances in Sexually Transmitted Diseases, ed. R.S.Morton and J.R.W.Harris, 357
  Skin Diseases in the Elderly, J.Verbov, 236
  Sunlight and Man. Normal and abnormal photobiologic responses, ed. T.B.Fitzpatrick, M. Pathak, L.Haber, M. Seiji and A.Kukita, 355
  The Physiology and Pathophysiology of the Skin. Vol. 3. *The Dermis and the Dendrocytes* ed. A.Jarrett, 606
  The Structure and Function of Skin, 3rd edition, W.Montagna and P.F.Parakkal, 355
  The Systemic Manifestations of Inflammatory Bowel Disease, ed. W.B.Luckash and R.B.Johnson, 609
  Viruskrankheiten der Haut, der Schleimhaute und des Genitales, T.Nasemann, with G.Schaeg and O.Schulta, 358
  Writing Scientific Papers in English. An ELSE-CIBA Foundation Guide for Authors, Maeve O'Connor and F.P.Woodford, 357
British Association of Dermatologists: Annual Meeting, Cambridge 1975—programme, summaries of papers, and case reports, Suppl. 11
Bullous diseases; international mortality from, since 1950 (summary), Suppl. 11, 17

Calcinosis universalis (case report), Suppl. 11, 45
Candida albicans infection in median rhomboid glossitis, 399
Carbohydrate metabolism and the HLA system; investigations of, in lichen planus (summary), Suppl. 11, 18
Carcinoid tumour of the bronchus, malignant: associated with erythema annulare centrifugum and white banding of the toe nails, 341
Carcinoma: intra-epidermal, of the eyelid margin, 239
  mucinous ovarian; associated with lichen myxoedematosus and Crohn's disease (case report), Suppl. 11, 34
β-Carotene and the photodermatoses, 721
Catecholamine and related compounds; effects of on the adenyl cyclase system in the epidermis, 29
Cell cultures and tumours of pigmented basal cell epithelioma; light and electron microscopic studies on, 361
Cell-mediated immunity: deficient, associated with multiple keratoacanthomas, 593
  in patients with warts, 553
Cells: epidermal cells, isolated, of guinea-pig: differential binding of antibody from the sera of patients with pemphigus and pemphigoid to, 425
  infiltrating cells, viable; method of liberating from dermal infiltrate, 271
  leukocyte migration inhibition test and complement fixation in measurement of cellular immunity and circulating antibody to herpes simplex virus, 539
  lymphocytes and amino acids; nickel binding to, 557
    prealbumin bearing; as indicator of hapten protein carrier in certain varieties of dermatitis, 11
  lymphoid cells; identification of in cutaneous infiltrate of skin reticuloses and lichen planus (summary), Suppl. 11, 16
  naevus cells and moles (summary), Suppl. 11, 15
Chicken pox, followed by morphoea (case report), Suppl. 11, 43
Chromatography, thin layer: and counter-current analysis in porphyrias, 277
  use of in separation of free porphyrins and porphyrin methyl esters, 291
Clofazimine in the treatment of leprosy (summary), Suppl. 11, 13
Coal tar fractions; evaluation of, for use in psoriasiform diseases, using the mouse tail test, 67
Colitis, ulcerative; and lichen planus, 465
Collagen: long-spacing; in skin biopsies from patients with lepromatous leprosy, 175
  skin collagen density and skin thickness; influence of age and sex on, 639
Colloid milium, pigmented, and ochronosis from hydroquinone bleaching creams in man (summary), Suppl. 11, 9; 613
Comment, 111, 231, 351, 721
Complement fixation and mixed leukocyte migration inhibition test in measurement of cellular immunity and circulating antibody to herpes simplex virus, 539
Correspondence, 353, 473, 605, 731
Corticosteroids: an improved method of assessing topical corticosteroid activity, 459
  corticosteroid preparations, proprietary topical; comparative bio-availability and activity of: vasoconstrictor assays on 31 ointments, 563
  nandrolone decanoate; percutaneous penetration of, 47
  triamcinolone acetonide (jet-injected) in treatment of granuloma annulare and necrobiosis lipoidica, 85
Counter-current analysis and thin layer chromatography in porphyrias, 277
Cowden disease, 329
Creams, bleaching, hydroquinone; causing ochronosis and pigmented colloid milium (summary), Suppl. 11, 9; 613
Crohn's disease, lichen myxoedematosus and mucinous ovarian carcinoma (case report), Suppl. 11, 34
Cyclophosphamide and prednisolone sodium phosphate; reactivation of *Herpesvirus hominis*

Cyclophosphamide (*cont.*)
  infection in mice treated with (summary), Suppl. 11, 24
  in the treatment of pemphigus, 573
Cysts, skin; bacteriology of (summary), Suppl. 11, 25
Cytophotometry, impulse; in psoriasis, 225

Dapsone; inhibition of lysosomal enzymes by, 471
Deafness: complicating mucous membrane pemphigoid, 337
  with urticaria and amyloidosis. Muckle Wells syndrome (case report), Suppl. 11, 46
Dermal infiltrate; method of liberating living cells from, 271
Dermatitis:
  contact: due to nickel, 187
  herpetiformis; studies of scratching during sleep, 297
  prealbumin bearing lymphocytes as an indicator of hapten protein carrier in certain varieties of, 11
Dermatology, International Committee of: report of meeting in Mexico City, 31 October–3 November 1974, 603
Dermis: focal hypoplasia of (case report), Suppl. 11, 70
  lipid deposition in, following renal transplant (case report), Suppl. 11, 59
Diazoxide, causing hypertrichosis, 707
Dithranol and a 'synthetic tar'; evaluation of as antipsoriatic treatments using the mouse tail test, 75
  treatment (simplified) for psoriasis, 205
Dixarit, rosacea, migraine (summary), Suppl. 11, 11
DNA, increased, and protein synthesis in the involved and uninvolved skin of patients with psoriasis (summary), Suppl. 11, 28
  skin test in systemic lupus erythematosus: clinical evaluation, 443;
  histological findings, 451
Drug eruption: D-penicillamine inducing pemphigus (summary), Suppl. 11, 12
Drugs, topical and systemic, in treatment of psoriasis; animal model for screening for potential use (summary), Suppl. 11, 27

Eczema: and acne; familial study (summary), Suppl. 11, 10
  atopic; studies of scratching during sleep, 297
  nummular; with staphylococcal sensitivity (case report), Suppl. 11, 40
Elaunin fibres in the secretory coil of human eccrine sweat glands, 623
Electron microscopy:
  electron microscopy of the sweat glands from the hairy skin of the beagle, 348
  fine structure of the elastic tendons in the human *arrector pili* muscle, 631
  intraendothelial tubular aggregates in experimental wounds, 165
  light and electron microscopic studies on tumours and cell cultures of pigmented basal cell epithelioma, 361
  long-spacing collagen in skin biopsies from patients with lepromatous leprosy (summary), Suppl. 11, 175
  naevus spilus: a light and electron microscopical study, Suppl. 11, 16
  ultrastructural localization and differentiation of membrane-bound ATP utilizing enzymes including adenyl cyclase in normal and psoriatic epidermis, 495
  ultrastructural study of elaunin fibres in the secretory coil of human eccrine sweat glands, 623
Enzymes:
  adenyl cyclase system in epidermis; effects of catecholamine and related compounds on, 29
  ATP-hydrolytic activity, membrane-bound: of normal and psoriatic epidermis, 496; of psoriatic epidermis, 469
  enzyme-cytochemical and immunocytological studies in the classification and differentiation of cutaneous lymphomas, 597
  epidermal energy metabolism in lichen planus, 667
  ester hydrolases of guinea-pig epidermis, 391
  glucose-6-phosphate dehydrogenase; activity in erythrocytes in lichen planus (letter), 353, 354
  glycosidases of guinea-pig epidermis, 1
  lipase, hyaluronidase and protease production from bacteria isolated from acne patients (summary), Suppl. 11, 26
  lysosomal enzymes; inhibition of by dapsone, 471
  peptide hydrolases in guinea-pig epidermis, 509
Epidermal cells, isolated, of guinea-pig; differential binding of antibody from the sera of patients with pemphigus and pemphigoid to, 425
Epidermis: effects of catecholamine and related compounds on the adenyl cyclase system in, 29
  effects of dermo–epidermal adhesion on the structure of, in the mouse, 417
  lysosomal hydrolases of. 1. Glycosidases, 1; 2. Ester hydrolases, 391; 3. Peptide hydrolases, 509
  normal and psoriatic; membrane-bound ATP activity in, 495
  psoriatic; levels of cyclic AMP in, 253
  psoriatic; membrane-bound ATP-hydrolytic activity of, 469
  structure and metabolic activity of in acromegaly (summary), Suppl. 11, 29
  transit times of, 649
Epidermolysis bullosa; controlled study of vitamin E treatment in (summary), Suppl. 11, 10
Epithelioma, basal cell, pigmented, 361
Erythema annulare centrifugum and white banding of the toe nails; associated with malignant carcinoid tumour of bronchus, 341
  nodosum from *Yersinia enterocolitica*, 719
Erythrocyte glucose-6-phosphate dehydrogenase in lichen planus (letter), 353, 354
Extremities, hypersensitive and insensitive: dermatological aspects, 151

Fibrin and immunoglobulin deposition in lichen planus; diagnostic significance of, 17

Folliculitis (case report), Suppl. 11, 49
  decalvans, recurrent; with keratosis pilaris (case report), Suppl. 11, 55
Food additives and aspirin: reactions to in patients with chronic urticaria, including the physical urticarias, 135
Fourier analysis of thermal sweating, 223
Fragrance ingredients; the safety of, 351
Fungal infections: *Candida albicans* in median rhomboid glossitis, 399
  tinea pedis, in sportsmen; efficacy of miconazole as topical treatment in, 79

Gingivitis, plasma cell (case report), Suppl. 11, 65
Glossitis, median rhomboid: candidiasis and not a developmental anomaly, 399
Glucose-6-phosphate dehydrogenase activity in erythrocytes in lichen planus (letter), 353, 354
Gout and xanthoma tuberosum (case report), Suppl. 11, 57
Graham-Little syndrome (follicular keratosis with cicatricial alopecia) (case report), Suppl. 11, 53
Granuloma annulare and necrobiosis lipoidica treated by jet injector, 85
  in Mauriac's syndrome (case report), Suppl. 11, 31

Hair fragment in the skin resembling larva migrans, 349
  nickel content of, 187
  tonics, proprietary; pigmentation of palms and scalp probably due to phenols and phenolic derivatives in, 718
Hapten protein carrier; prealbumin bearing lymphocytes as indicator of, in certain varieties of dermatitis, 11
Hemiatrophy, facial (case report), Suppl. 11, 65
Herpes gestationis; immunopathological findings in Suppl. 11, 17
  simplex; immunity in (summary), Suppl. 11, 15
    virus; cellular immune responses to, 539
Herpesvirus hominis; reactivation of infection in mice treated with cyclophosphamide and prednisolone sodium phosphate (summary), Suppl. 11, 24
Hexachlorophane; absorption of from steroid ointment, 209
HLA system and carbohydrate metabolism; investigations of, in lichen planus (summary), Suppl. 11, 18
Hodgkin's disease in a patient with follicular and dermal mucinosis, 581
Hyaluronidase, lipase and protease production from bacteria isolated from acne patients (summary), Suppl. 11, 26
Hydrolases, lysosomal, of the epidermis. 1. Glycosidases, 1. 2. Ester hydrolases, 391 3. Peptide hydrolases, 509
Hydroquinone bleaching creams, causing ochronosis and pigmented colloid milium in man (summary), Suppl. 11, 9; 613
Hyperlipoproteinaemia and multiple myeloma associated with plane xanthomata, 407
Hypermelanosis; treatment of with 4-isopropylcatechol (summary), Suppl. 11, 9
Hyperthyroidism and urticaria, 97
Hypertrichosis due to diazoxide, 707
Hypoplasia, dermal, focal (case report), Suppl 11, 70

Immunology:
  cell-mediated immunity, deficient; associated with multiple keratoacanthomas, 593
    responses in patients with warts, 553
  cellular immune responses to herpes simplex virus, 539
  deficiency of circulating IgA and IgM in adult patients with lichen planus, 25
  diagnostic significance of immunoglobulin and fibrin deposition in lichen planus, 17
  differential binding of antibody from the sera of patients with pemphigus and pemphigoid to isolated guinea-pig epidermal cells, 425
  DNA skin test in systemic lupus erythematosus: clinical evaluation, 443; histological findings, 451
  eosinophilic spongiosis; clinical, histological and immunofluorescent correlation (summary), Suppl. 11, 13
  immunity in herpes simplex (summary), Suppl. 11, 15
  immunocytological and enzyme-cytochemical studies in the classification and differentiation of cutaneous lymphomas, 597
  immunohistological studies, with anti-connective tissue and anti-immunoglobulin antisera, of the skin in lupus erythematosus and scleroderma, 431
  immunological responses of patients with psoriasis and effect of treatment with methotrexate, 659
  immunopathological findings in herpes gestationis (summary), Suppl. 11, 17
    study of coexistence of lichen planus and bullous pemphigoid, 313
  immunosuppression and skin infection, 115
  in vitro assay of immunity to human wart antigen, 545
  investigations of carbohydrate metabolism and the HLA system in lichen planus (summary), Suppl. 11, 18
  prealbumin bearing lymphocytes as indicator of hapten protein carrier in certain varieties of dermatitis, 11
  significance of a positive cutaneous immunofluorescence test in systemic lupus erythematosus, 531
  wart immunity, autoantibodies and Australia antigen in sarcoidosis, 717
Infection; natural antibiosis among skin bacteria as primary defence against, 487
Irish Dermatological Society, 729
Irritants, primary; 100 twin pairs patch tested with, 145

4-Isopropylcatechol in the treatment of hypermelanosis (summary), Suppl. 11, 9
Itch; new method for measurement of and of the response to treatment, 303

Jaundice; vitiligo and, 229
Jet injector; use of in treatment of granuloma annulare and necrobiosis lipoidica, 85

Keratin hydration and sebum excretion rate (summary), Suppl. 11, 27
Keratitis in rosacea treated by tetracycline; follow-up study (summary), Suppl. 11, 12; 577
Keratoacanthoma in a Bantu patient, 699
  s, multiple: associated with deficient cell-mediated immunity, 593
Keratosis, follicular (case report), Suppl. 11, 48
    with cicatricial alopecia (case report), Suppl. 11, 53
  pilaris with recurrent folliculitis decalvans (case report), Suppl. 11, 55

Larva migrans; hair fragment in the skin resembling, 349
Leishmaniasis, cutaneous; tissue sampling with a dental broach in, 43
Leprechaunism, 587
Leprosy, lepromatous: clofazimine in the treatment of (summary), Suppl. 11, 13
  long-spacing collagen in skin biopsies from patients with, 175
Leukocyte migration inhibition test and complement fixation in measurement of cellular immunity and circulating antibody to herpes simplex virus, 539
Lichen myxoedematosus, Crohn's disease and mucinous ovarian carcinoma (case report), Suppl. 11, 34
  planus: and bullous pemphigoid; coexistence of, 313
    and skin reticuloses; comparison of nature of dermal infiltrate in, by use of liberated living cell method, (summary) Suppl. 11, 16; 271
    and ulcerative colitis, 465
    deficiency of circulating IgA and IgM in adult patients with, 25
    diagnostic significance of immunoglobulin and fibrin deposition in, 17
    erythrocyte glucose-6-phosphate dehydrogenase in (letter), 353, 354
    investigations of carbohydrate metabolism and the HLA system (summary), Suppl. 11, 18
    studies of scratching during sleep, 297
    studies on the energy metabolism in, 667
    transplantation of bone marrow followed by lichen planus-like eruption, 675
  sclerosus et atrophicus, generalized bullous and haemorrhagic; treated with ACTH, 215
Light reaction, persistent, in quindoxin and quinine photosensitivity; study of (summary), Suppl. 11, 21
Lipase, hyaluronidase and protease production from bacteria isolated from acne patients (summary), Suppl. 11, 26
Lipid deposition in the dermis following renal transplant (case report), Suppl. 11, 59
Livedo reticularis, 519
Liver failure; hazards of steroid therapy in, 183
Lupus erythematosus and scleroderma; immunohistological studies of the skin in, 431
  discoid (case report), Suppl. 11, 38
  systemic; DNA skin test in; clinical evaluation, 443; histological findings, 451
    significance of a positive cutaneous immunofluorescence test in, 531
Lymphocytes and amino acids; nickel binding to, 557
  prealbumin bearing; as indicator of hapten protein carrier in certain varieties of dermatitis, 11
Lymphoid cells; identification of in cutaneous infiltrate of skin reticuloses and lichen planus (summary), Suppl. 11, 16
Lymphomas, cutaneous; classification and differentiation of, 597

Mastocytosis, pseudoxanthomatous, 91
Mauriac's syndrome; granuloma annulare in (case report), Suppl. 11, 31
Methotrexate: immunological responses of patients with psoriasis and effect of treatment with, 659
  pneumonitis in a psoriatic treated with, 107
  reactivation of scabies rash by, 219
  weekly oral, in severe psoriasis; prospective study of the effects of, on liver biopsy, 321
8-(Methylthio)cyclic AMP-^{35}S, topically applied in dogs and humans; absorption and biotransformation of, 379
Miconazole; efficacy of in the topical treatment of tinea pedis in sportsmen, 79
Microbiology review: immunosuppression and skin infection, 115
Micro-organisms of skin; dispersal of, 477
Migraine, rosacea, Dixarit (summary), Suppl. 11, 11
Moles, in relation to naevus cells (summary), Suppl. 11, 15
Mongolism; cutaneous aspects of (summary), Suppl. 11, 14
Morphoea arising in a hypoplastic breast (case report), Suppl. 11, 44
  following chicken pox (case report), Suppl. 11, 43
Mucinosis, follicular and dermal, and mucinorrhoea associated with Hodgkin's disease, 581
Muckle Wells syndrome (urticaria, amyloidosis, deafness) (case report), Suppl. 11, 46
Muscle, *arrector pili*, human; fine structure of elastic tendons in, 631
Mycosis fungoides with necrotizing angiitis (case report), Suppl. 11, 62
Myeloma, multiple, and hyperlipoproteinaemia, associated with plane xanthomata, 407

Naevus cells in relation to moles (summary), Suppl. 11, 15
  spilus: a light and electron microscopical study (summary), Suppl. 11, 16
  superficialis lipomatosus, 121

Nails, finger: growth in peripheral pustulosis (summary), Suppl. 11, 24
  toe: white banding of, and erythema annulare centrifugum; associated with malignant carcinoid tumour of the bronchus, 341
Nandrolone decanoate; percutaneous penetration of, 47
Necrobiosis lipoidica and granuloma annulare treated by jet injector, 85
  diabeticorum involving scalp and face (case), 729
Necrosis, recurrent, of the skin and the mouth, 101
News and notices, 238, 486, 611, 732
Nickel: hypersensitivity, 557
  levels in tissue and nickel dermatitis—I. Nickel in hair, 187
  sensitivity resembling bullous pemphigoid (case report), Suppl. 11, 41

Obituaries:
  Glyn-Hughes, F., 728
  Goldsmith, W. N., 725
  Kindler, Theresa, 601
  Sklarz, Ernest, 601
Ochronosis and pigmented colloid milium from hydroquinone bleaching creams in man (summary), Suppl. 11, 9; 613
Ointment, steroid; absorption of hexachlorophane from, 209
Ophionyssus natricis snake mite; human infestation by, 695

Palmar ridge appearances in normal newborn infants and ridge appearance in relation to eccrine sweating, 645
Parasites: human infestation by *Ophionyssus natricis* snake mite, 695
Patch testing: 100 twin pairs patch tested with primary irritants, 145
Pemphigoid: and pemphigus; differential binding of antibody from the sera of patients with, to isolated guinea-pig epidermal cells, 425
  bullous; coexisting with lichen planus, 313
    simulated by nickel sensitivity (case report), Suppl. 11, 41
  mucous membrane; deafness as a complication of, 337
Pemphigus: and pemphigoid; differential binding of antibody from the sera of patients with, to isolated guinea-pig epidermal cells, 425
  eosinophilic spongiosis in (summary), Suppl. 11, 13
  induced by D-penicillamine (summary), Suppl. 11, 12
  treated with cyclophosphamide, 573
D-Penicillamine, inducing pemphigus (summary), Suppl. 11, 12
Percutaneous penetration of nandrolone decanoate, 47
Periodontal membrane in systemic sclerosis (summary), Suppl. 11, 23
Phenols and phenolic derivatives in proprietary hair tonics; probable cause of pigmentation of palms and scalp, 718
Photodermatoses and β-carotene, 721
Photomicrographs; choice and use of equipment for, 231
Photosensitivity, due to quindoxin and quinine; study of persistent light reaction in (summary), Suppl. 11, 21
  submicroscopic events during acute flare of in erythropoietic protoporphyrias (letter), 605
Pigmentation: acquired; of skin folds, with histological picture of seborrhoeic wart, 713
  demarcation lines—comparison of Negroes with Japanese, 371
  of palms and scalp, probably due to phenols and phenolic derivatives in proprietary hair tonics, 718
Pili torti (case report), Suppl. 11, 64
Pilosebaceous unit; pathogenic interpretation of histologic changes in, in trichotillomania (summary), Suppl. 11, 21
Pityriasis alba, follicular (case report), Suppl. 11, 51
Plasma uroporphyrin levels in cutaneous hepatic porphyria; effect of treatment on, 37
Pneumonitis in a methotrexate-treated psoriatic, 107
Polychondritis, relapsing (case report), Suppl. 11, 67
Polyvinyl chloride; preliminary report of vascular abnormality occurring in men engaged in manufacture of (summary), Suppl. 11, 22
Porphyria, cutaneous hepatic; effect of treatment on plasma uroporphyrin levels in, 37
  s; thin layer chromatography and counter-current analysis in, 277
Porphyrins and porphyrin methyl esters; use of thin layer chromatography in separation of, 291
Potash soap; patch test reactions to, in twin pairs, 145
Prednisolone sodium phosphate and cyclophosphamide; reactivation of *Herpesvirus hominis* infection in mice treated with (summary), Suppl. 11, 24
Protease, lipase and hyaluronidase production from bacteria isolated from acne patients (summary), Suppl. 11, 26
Protein synthesis and increased DNA in the involved and uninvolved skin of patients with psoriasis (summary), Suppl. 11, 28
Protoporphyria, erythropoietic: submicroscopic events during the acute photosensitivity flare (letter), 605
Protothecosis, cutaneous (letter), 473
Pruritus vulvae: treatment by multiple intradermal alcohol injections, 201
Psoriasiform diseases; evaluation of coal tar fractions for use in, using the mouse tail test, 67
Psoriasis: animal model for screening topical and systemic drugs for potential use in treatment of (summary), Suppl. 11, 27
  epidermal levels of cyclic AMP in, 253
  evaluation of dithranol and a 'synthetic tar' as treatment for, using the mouse tail test, 75
  immunological responses of patients with, and effect of treatment with methotrexate, 659
  impulse-cytophotometry in, 225
  increased DNA and protein synthesis in the involved

Psoriasis (*cont.*)
        and uninvolved skin of patients with (summary), Suppl. 11, 28
    membrane-bound ATP-hydrolytic activity of epidermis in, 469
    pneumonitis in a methotrexate-treated patient with, 107
    psoriatic and normal skin; membrane-bound ATP activity in, 495
    pustular; juvenile generalized (case report), Suppl. 11, 40
    severe; prospective study of the effects of weekly oral methotrexate on liver biopsy, 321
    simplified dithranol treatment for, 205
    studies of scratching during sleep, 297
    the psoriasis sufferer in the community 111
Purpura, anaphylactoid; caused by tartrazine and benzoates (case report), Suppl. 11, 61
Pustulosis, peripheral; fingernail growth in (summary), Suppl. 11, 24
Pyoderma gangrenosum (case report), Suppl. 11, 31

Quindoxin and quinine photosensitivity; study of persistent light reaction in (summary), Suppl. 11, 21

Renal transplant; followed by lipid deposition in the dermis (summary), Suppl. 11, 59
Reticuloses of the skin and lichen planus: comparison of nature of dermal infiltrate in, by use of liberated living cell method (summary), Suppl. 11, 16; 271
Rosacea: associated with male pattern baldness, 63
    migraine and treated with Dixarit (summary), Suppl. 11, 11
    tetracycline-treated; follow-up with special reference to rosacea keratitis (summary), Suppl. 11, 12; 577

Saline, sterile normal (jet-injected), in treatment of granuloma annulare and necrobiosis lipoidica, 85
Sarcoidosis (case report), Suppl. 11, 68
    wart immunity, autoantibodies and Australia antigen in, 717
Sarcoma, epithelioid, of finger pulp (case report), Suppl. 11, 71
Scabies rash; reactivation of by methotrexate, 219
Scleroderma and lupus erythematosus; immunohistological studies of the skin in, 431
    arterial thrombosis in, 683
Scleromyxoedema (case report), Suppl. 11, 36
Sclerosis, systemic; immunohistological studies of the skin in, 431
    periodontal membrane in (summary), Suppl. 11, 23
Scratching, as a measurement of itch, 303
    during sleep; further studies, 297
Sebum excretion rate and keratin hydration (summary), Suppl. 11, 27
    investigations: a new absorbent paper, 347
Sensitization to nickel, 557
    resembling bullous pemphigoid (case report), Suppl. 11, 41
    staphylococcus, associated with nummular eczema (case report), Suppl. 11, 40
Skin: and mouth; recurrent necrosis of, 101
    disorders in the urban black population of South Africa; survey of, 259
    infection and immunosuppression, 115
    micro-organisms; dispersal of (review), 477
    physiological and environmental control of Gram negative bacteria on, 191
    thickness, skin collagen and density; influence of age and sex on, 639
Snake mite (*Ophionyssus natricis*); human infestation by, 695
Sodium lauryl sulphate; patch test reactions to, in twin pairs, 145
Spongiosis, eosinophilic: clinical, histological and immunofluorescent correlation (summary), Suppl. 11, 13
Staphylococcus; sensitivity to, associated with nummular eczema (case report), Suppl. 11, 40
Steroid therapy; hazards of, in hepatic failure, 183
Stratum corneum; temperature dependence of water content of, 159
Sweat glands, eccrine, human; ultrastructural study of elaunin fibres in the secretory coil of, 623
    from the hairy skin of the beagle; electron microscopy of, 348
Sweating, eccrine; palmar ridge appearances in normal newborn infants and ridge appearance in relation to, 645
    thermal: Fourier analysis of, 223
Syphilis, secondary: clinico-pathological review, 53

Tar oil acids, high boiling; evaluation of for use in psoriasiform diseases, using the mouse tail test, 67
    synthetic, and dithranol; evaluation of as antipsoriatic treatments using the mouse tail test, 75
Tartrazine and benzoates causing anaphylactoid purpura (case report), Suppl. 11, 61
Temperature dependence of water content of stratum corneum, 159
Tendons, elastic; fine structure of, in the human *arrector pili* muscle, 631
Tetracycline in the treatment of rosacea; follow-up, with special reference to rosacea keratitis (summary), Suppl. 11, 12; 577
Thrombosis, arterial; in scleroderma, 683
Tinea pedis; efficacy of miconazole in the topical treatment of, in sportsmen, 79
Tissue sampling with a dental broach, in cutaneous leishmaniasis, 43
Treatment:
    ACTH in lichen sclerosus et atrophicus, 215
    alcohol injections, multiple intradermal, in pruritus vulvae, 201
    clofazimine in leprosy (summary), Suppl. 11, 13
    cyclophosphamide in pemphigus, 573
    dithranol (simplified) in psoriasis, 205
    Dixarit in rosacea (summary), Suppl. 11, 11

Treatment (*cont.*)
  4-isopropylcatechol in hypermelanosis (summary), Supp. 11, 9
  methotrexate in psoriasis, 321
    effect of treatment on immunological responses of patients, 659
  miconazole, topical, in tinea pedis, 79
  steroids in hepatic failure; hazards of, 183
  tetracycline in rosacea; follow-up with special reference to rosacea keratitis (summary), Suppl. 11, 12; 577
  triamcinolone acetonide or sterile normal saline (jet-injected) in granuloma annulare and necrobiosis lipoidica, 85
  vitamin E treatment in epidermolysis bullosa (summary), Suppl. 11, 10
Triamcinolone acetonide (jet-injected) in treatment of granuloma annulare and necrobiosis lipoidica, 85
Trichotillomania: a pathogenic interpretation of histologic changes in the pilosebaceous unit (summary), Suppl. 11, 21
Trypsin, in the preparation of isolated epidermal cells, 425
Tubular aggregates, intraendothelial; in experimental wounds, 165

Uroporphyrin levels in plasma in cutaneous hepatic porphyria; effect of treatment on, 37
Urticaria, amyloidosis and deafness. Muckle Wells syndrome (case report), Suppl. 11, 46
  and hyperthyroidism, 97
  chronic; challenge test battery in (summary), Suppl. 11, 19
  (including physical urticarias); reactions to aspirin and food additives in patients with, 135
  studies of scratching during sleep, 297
s, physical; induced tolerance in (letter), 473

Vascular abnormality occurring in men engaged in manufacture of polyvinyl chloride; preliminary report (summary), Suppl. 11, 22
Vasoconstrictor assays in comparison of bio-availability and activity of proprietary topical corticosteroid preparations, 563
Vitamin E treatment in epidermolysis bullosa; controlled study (summary), Supp. 11, 10
Vitiligo and jaundice, 229

Wart antigen, human; *in vitro* assay of immunity to, 545
  immunity, autoantibodies and Australia antigen in sarcoidosis, 717
  seborrhoeic; acquired pigmentation of skin folds with histological picture of, 713
  cell-mediated immune responses in patients with, 553
Water content of stratum corneum; temperature dependence of, 159
Wounds, experimental: intraendothelial tubular aggregates in, 165

Xanthoma tuberosum and gout (case report), Suppl. 11, 57
Xanthomata, plane; associated with multiple myeloma and hyperlipoproteinaemia, 407

Yersinia enterocolitica, causing erythema nodosum, 719

AUTHORS

ABELL, E., MARKS, R. and WILSON JONES, E., secondary syphilis: a clinico-pathological review, 53
ABELL, E., PRESBURY, D.G.C., MARKS, R. and RAMNARAIN, D., the diagnostic significance of immunoglobulin and fibrin deposition in lichen planus, 17
ABELL, E., SPARROW, G. and: granuloma annulare and necrobiosis lipoidica treated by jet injector, 85
ADACHI, K., HALPRIN, K.M., LEVINE, V.; YOSHIKAWA, K. and: is the cyclic AMP in psoriatic epidermis low?, 253
ADACHI, K., HALPRIN, K.M., LEVINE, V.; YOSHIKAWA, K. and: the effects of catecholamine and related compounds on the adenyl cyclase system in the epidermis, 29
ADAM, KIRSTINE, PATERSON, W.D., OSWALD, I.; SAVIN, J.A. and: further studies of scratching during sleep, 297
ADAMS, ROWAN H., MAIN, R.A. and MARSDEN, R.A., a controlled study of vitamin E treatment in epidermolysis bullosa (summary), Suppl. 11, 10
AKERS, W.A., LINAMEN, C.E., JONES, H.E.; SPENCER, T.S. and: temperature dependence of water content of stratum corneum, 159
ALLEN, B.R., PARKER, SHEENA, THOMPSON, G.G., MOORE, M.R., DARBY, F.J. and HUNTER, J.A.A., effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria, 37
ALMEIDA, J., HERRERO, C., SMITH, S.G., BELCHER, R.V.; PIÑOL AGUADÉ, J. and: thin layer chromatography and counter-current analysis in porphyrias, 277
AMOS, H.E.; BRIGDEN, W.D. and: the differential binding of antibody from the sera of patients with pemphigus and pemphigoid to isolated guinea-pig epidermal cells, 425
ARDALAN, B., DOWD, P.M.; EVERALL, J.D. and: unusual cutaneous associations of a malignant carcinoid tumour of the bronchus—erythema annulare centrifugum and white banding of the toe nails, 341

Azulay, R.D., personal experience with clofazimine in the treatment of leprosy (summary), Suppl. 11, 13

Baker, H., Landells, J.W., Levene, G.M.; Warin, A.P. and: a prospective study of the effects of weekly oral methotrexate on liver biopsy, 321

Baldock, N.E. and Catterall, M.D., erythema nodosum from *Yersinia enterocolitica*, 719

Barnes, H., Gaylarde, P.M., Brock, A.P. and Sarkany, I., an improved method of assessing topical corticosteroid activity, 459

Barry, B.W. and Woodford, R., comparative bioavailability and activity of proprietary topical corticosteroid preparations: vasoconstrictor assays on thirty-one ointments, 563

Bartlett, R.G., Dogliotti, M., Rabinowitz, L.; Hartdegen, R.G. and: leprechaunism, 587

Bauer, F.W. and De Grood, R.M., impulse cytophotometry in psoriasis, 225

Beck, G.A., follicular pityriasis alba (case report), Suppl. 11, 51

Beck, G.A.; Singh, J. and: folliculitis (case report), Suppl. 11, 49

Beck, G.A.; Singh, J. and: morphoea following chicken pox (case report), Suppl. 11, 43

Belcher, R.V., Herrero, C., Almeida, J., Smith, S.G.; Piñol Aguadé, J. and: thin layer chromatography and counter-current analysis in porphyrias, 277

Bentley-Phillips, C.B. and Marks, R., are moles just naevus cells? (summary), Suppl. 11, 15

Benveniste, M., Lessana-Leibowitch, M.; Hewitt, J. and: pemphigus induced by D-penicillamine (summary), Suppl. 11, 12

Bergers, A.M.S., Van Hasselt, P.; Cotton, D.W.K. and: electron microscopy of the sweat glands from the hairy skin of the beagle, 348

Bittencourt-Sampaio, S., Guerra Rodrigo, F.; Cotta-Pereira, G. and: ultrastructural study of elaunin fibres in the secretory coil of human eccrine sweat glands, 623

Black, M.M.; Carruthers, J.A. and: immunopathological findings in herpes gestationis (summary), Suppl. 11, 17

Black, M.M., Delaney, T.J.; Knight, A.G. and: eosinophilic spongiosis; a clinical, histological and immunofluorescent correlation (summary), Suppl. 11, 13

Black, M.M., McVitie, Eva; Shuster, S. and: the influence of age and sex on skin thickness, skin collagen and density, 639

Bleehen, S.S., pigmented basal cell epithelioma, 361

Bleehen, S.S., the treatment of hypermelanosis with 4-isopropylcatechol (summary), Suppl. 11, 9

Bowen, H.J.M., Comaish, J.S., Samitz, M.H.; Katz, S.A. and: tissue nickel levels and nickel dermatitis. I. Nickel in hair, 187

Bradstreet, C.M.P., De Bats, A., Park, J.R., Rhodes, E.L.; Gange, R. W. and: cellular immunity and circulating antibody to herpes simplex virus in subjects with recurrent herpes simplex lesions and controls as measured by the mixed leukocyte migration inhibition test and complement fixation, 539

Bradstreet, C.M.P., De Bats, A., Park, J.R., Rhodes, E.L.; Gange, R.W. and: immunity in herpes simplex (summary), Suppl. 11, 15

Braun-Falco, O.; Burg, G. and: classification and differentiation of cutaneous lymphomas. Enzyme-cytochemical and immunocytological studies, 597

Bridges, J.M., Morris, T.C.M.; Burrows, D. and: reactivation of scabies rash by methotrexate, 219

Brigden, W.D. and Amos, H.E., the differential binding of antibody from the sera of patients with pemphigus and pemphigoid to isolated guinea-pig epidermal cells, 425

Britten, A.Z.; Wrench, Rosanne, and: evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test. III. High boiling tar oil acids, 67

Britten, A.Z.; Wrench, Rosanne, and: evaluation of dithranol and a 'synthetic tar' as anti-psoriatic treatments using the mouse tail test, 75

Brock, A.P., Gaylarde, P.M., Sarkany, I.; Barnes, H. and: an improved method of assessing topical corticosteroid activity, 459

Brostoff, J.; Levantine, A. and: immunological responses of patients with psoriasis and the effect of treatment with methotrexate, 659

Bullough, W.S. and Deol, Johanna U.R., dermo-epidermal adhesion and its effect on epidermal structure in the mouse, 417

Burg, G. and Braun-Falco, O., classification and differentiation of cutaneous lymphomas. Enzyme-cytochemical and immunocytological studies, 597

Burnett, J.W., Goldner, R. and Calton, G.J., Cowden disease, 329

Burrows, D., Bridges, J.M. and Morris, T.C.M., reactivation of scabies rash by methotrexate, 219

Burton, J.L., Holti, G., Wright, V.; Cunliffe, W.J. and: hazards of steroid therapy in hepatic failure, 183

Burton, J.L., Schutt, W.H. and Caldwell, I.W., hypertrichosis due to diazoxide, 707

Bussel, Anette, Gluckman, Eliane, Didierjean, Liliane, Puissant, A.; Saurat, J.H. and: the lichen planus-like eruption after bone marrow transplantation, 675

Bye, P.G.T., Morison, W. and Rhodes, Ellen L., the absorption of hexachlorophane from Ultralanum ointment, 209

Byrne, J.P.N.; Dawber, R.P.R. and: fingernail growth in peripheral pustulosis: case report (summary), Suppl. 11, 24

Byrom, N.A., Hayes, J.P.; Tan, R.S.-H. and: a method of liberating living cells from the dermal infiltrate, 271

Byrom, N.A., Hayes, J.P.; Tan, R.S.-H. and: identification of lymphoid cells in the cutaneous infiltrate of skin reticuloses and lichen planus (summary), Suppl. 11, 16

Caldwell, I.W., Schutt, W.H.; Burton, J.L. and: hypertrichosis due to diazoxide, 707
Calton, G.J., Goldner, R.; Burnett, J.W. and: Cowden disease, 329
Carruthers, J.A. and Black, M.M., immunopathological findings in herpes gestationis (summary), Suppl. 11, 17
Catterall, M.D.; Baldock, N.E. and: erythema nodosum from *Yersinia entcrocolitica*, 719
Champion, R.H., facial hemiatrophy (case report), Suppl. 11, 65
Champion, R.H., focal dermal hypoplasia (case report), Suppl. 11, 70
Champion, R.H., urticaria, amyloidosis and deafness. Muckle Wells syndrome (case report), Suppl. 11, 46
Champion, R.H.; Goldin, D. and: follicular keratoses (case report), Suppl. 11, 48
Champion, R.H.; Goldin, D. and: morphoea arising in a hypoplastic breast (case report), Suppl. 11, 44
Champion, R.H.; Kubba, R. and: anaphylactoid purpura caused by tartrazine and benzoates (case report), Suppl. 11, 61
Champion, R.H.; Kubba, R. and: calcinosis universalis (case report), Suppl. 11, 45
Champion, R.H.; Kubba, R. and: nickel sensitivity resembling bullous pemphigoid (case report), Suppl. 11, 41
Champion, R.H.; Kubba, R. and: scleromyxoedema (case report), Suppl. 11, 36
Champion, R.H. and Nicholls, Anne: relapsing polychondritis (case report), Suppl. 11, 67
Champion, R.H., Parish, W.E.; Welbourn, Esther, and: nummular eczema with staphylococcal sensitivity (case report), Suppl. 11, 40
Clanachan, I.; Foreman, M.I. and: the percutaneous penetration of nandrolone decanoate, 47
Claudy, A. and Thivolet, J., multiple keratoacanthomas: association with deficient cell mediated immunity, 593
Coles, R.B. and Ryan, T.J., the psoriasis sufferer in the community, 111
Comaish, J.S., Bowen, H.J.M., Samitz, M.H.; Katz, S.A. and: tissue nickel levels and nickel dermatitis. I. Nickel in hair, 187
Cooke, B.E.D., median rhomboid glossitis. Candidiasis and not a developmental anomaly, 399
Copeman, P.W.M., livedo reticularis, 519
Cormane, R.H., Hamerlinck, F.; Husz, S. and: prealbumin bearing lymphocytes as an indicator of hapten protein carrier in certain varieties of dermatitis, 11
Cotta-Pereira, G., David-Ferreira, J.F.; Guerra Rodrigo, F. and: the fine structure of the elastic tendons in the human arrector pili muscle, 631
Cotta-Pereira, G., Guerra Rodrigo, F. and Bittencourt-Sampaio, S., ultrastructural study of elaunin fibres in the secretory coil of human eccrine sweat glands, 623
Cotton, D.W.K., De Jongh, G.J. and Thiele, F.A.J., Fourier analysis of thermal sweating, 223
Cotton, D.W.K. and Van Der Staak, W.B.J.M., erythrocyte glucose-6-phosphate dehydrogenase in lichen planus (letter), 354
Cotton, D.W.K., Van Hasselt, P. and Bergers, A.M.S., electron microscopy of the sweat glands from the hairy skin of the beagle, 348
Cotton, R.E., Kinmont, P.D.C., Wynn Williams, D., Wroughton, M.A.; McCallum, D.I. and: intra-epidermal carcinoma of the eyelid margin, 239
Cowen, T.C.; Eady, R.A.J. and: naevus spilus: a light and electron microscopical study (summary), Suppl. 11, 16
Craig, J.A., Lang, W.; Thomson, J. and: deafness complicating mucous membrane pemphigoid, 337
Cudworth, A.G., Vickers, C.F.H.; Lowe, N.J. and: lichen planus: investigations of carbohydrate metabolism and the HLA system (summary), Suppl. 11, 18
Cunliffe, W.J., Burton, J.L., Holti, G. and Wright, V., hazards of steroid therapy in hepatic failure, 183
Cunliffe, W.J., Holland, K.T.; Roberts, C.D. and: lipase, hyaluronidase and protease production from bacteria isolated from acne patients (summary), Suppl. 11, 26
Cunliffe, W.J., Perera, W.D.H., Williams, Margaret, Williams, Susan and Tan, Giaw, keratin hydration and sebum excretion rate (summary), Suppl. 11, 27
Cunliffe, W.J.; Tan, S.G. and: high doses of ascorbic acid (letter), 731
Cunliffe, W.J., Tan, S.G. and Tebbs, E., rosacea, migraine, Dixarit (summary), Suppl. 11, 11
Cunliffe, W.J., Williams, S.M. and Tan, S.G., sebum excretion rate investigations: a new absorbent paper, 347

Daneshbod, K.; Griffiths, W.A.D. and: pseudoxanthomatous mastocytosis, 91
Dantzig, P.I., Mauro, Jacqueline, Rayhanzadeh, S. and Rodofsky, U.H., the significance of a positive cutaneous immunofluorescence test in systemic lupus erythematosus, 531
Darby, F.J., Parker, Sheena, Thompson, G.G., Moore, M.R., Hunter, J.A.A.; Allen, B.R. and: effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria, 37
David-Ferreira, J.F., Cotta-Pereira, G.; Guerra Rodrigo, F. and: the fine structure of the elastic tendons in the human arrector pili muscle, 631
Dawber, R.P.R. and Byrne, J.P.N., fingernail growth in peripheral pustulosis: case report (summary), Suppl. 11, 24
De Bats, A., Park, J.R., Bradstreet, C.M.P., Rhodes, E.L.; Gange, R.W. and: cellular immunity and circulating antibody to herpes simplex virus in subjects with recurrent herpes simplex lesions and controls as measured by the mixed leukocyte migration inhibition test and complement fixation, 539
De Bats, A., Park, J.R., Bradstreet, C.M.P., Rhodes, E.L.; Gange, R.W. and: immunity in herpes simplex (summary), Suppl. 11, 15

De Grood, R.M.; Bauer, F.W. and: impulse cytophotometry in psoriasis, 225

De Jongh, G.J., Thiele, F.A.J.; Cotton, D.W.K. and: Fourier analysis of thermal sweating, 223

Delaney, T.J., Black, M.M.; Knight, A.G. and: eosinophilic spongiosis; a clinical, histological and immunofluorescent correlation (summary), Suppl. 11, 13

Deol, Johanna U.R.; Bullough, W.S. and: dermo-epidermal adhesion and its effect on epidermal structure in the mouse, 417

Di Silverio, A. and Serri, F., generalized bullous and haemorrhagic lichen sclerosus et atrophicus, 215

Didierjean, Liliane, Gluckman, Eliane, Bussel, Anette, Puissant, A.; Saurat, J.H. and: the lichen planus-like eruption after bone marrow transplantation, 675

Doeglas, H.M.G., reactions to aspirin and food additives in patients with chronic urticaria, including the physical urticarias, 135

Dogliotti, M., survey of skin disorders in the urban black population of South Africa, 259

Dogliotti, M., Mars, P.W., Rabson, A.R. and Rippey, J.J., cutaneous protothecosis (letter), 473

Dogliotti, M., Rabinowitz, L., Bartlett, R.G.; Hartdegen, R.G. and: leprechaunism, 587

Dowd, P.M., Ardalan, B.; Everall, J.D. and: unusual cutaneous associations of a malignant carcinoid tumour of the bronchus—erythema annulare centrifugum and white banding of the toe nails, 341

Dreyfuss, J., Mischler, T.W., Neiss, E.S., Shaw, J.M. and Schreiber, E.C., absorption and biotransformation of topically applied 8-(methylthio) cyclic AMP, 379

Du Vivier, A. and Stoughton, R.B., an animal model for screening topical and systemic drugs for potential use in the treatment of psoriasis using a new, rapid and simple technique of extracting DNA from epidermis involving hydroxyapatite column chromatography (summary), Suppl. 11, 27

Duncan, W.C., Knox, J.M.; McBride, M.E. and: physiological and environmental control of Gram negative bacteria on skin, 191

Dutz, W.; Griffiths, W.A.D. and: repeated tissue sampling with a dental broach. A trial in cutaneous leishmaniasis, 43

Eady, R.A.J. and Cowen, T.C., naevus spilus: a light and electron microscopical study (summary), Suppl. 11, 16

Eady, R.A.J. and Odland, G.F., intraendothelial tubular aggregates in experimental wounds, 165

Edwards, Rosa P., long-spacing collagen in skin biopsies from patients with lepromatous leprosy, 175

Elsahy, N.I., recurrent necrosis of the skin and the mouth, 101

Evans, D.B., Roberts, S.O.B.; Goldin, D. and: lipid deposition in the dermis following renal transplant (case report), Suppl. 11, 59

Evans, N.T.S., Naylor, P.F.D. and Sayers, B.McA., fluctuations in the blood flow of forearm skin (summary), Suppl. 11, 25

Everall, J.D., Dowd, P.M. and Ardalan, B., unusual cutaneous associations of a malignant carcinoid tumour of the bronchus—erythema annulare centrifugum and white banding of the toe nails, 341

Ewen, S.W.B.; Stankler, L. and: Hodgkin's disease in a patient with follicular and dermal mucinosis and spontaneous ooze of mucin (mucinorrhoea), 581

Felix, R. and Shuster, S., a new method for the measurement of itch and the response to treatment, 303

Fierens, F., Pierard, G.E.; Lachapelle, J.M. and: trichotillomania: a pathogenic interpretation of histologic changes in the pilosebaceous unit (summary), Suppl. 11, 21

Findlay, G.H., ochronosis and colloid milium from hydroquinone in man: 50 cases, with pathology and electron microscopy (summary), Suppl. 11, 9

Findlay, G.H., Morrison, J.G.L. and Simson, I.W., exogenous ochronosis and pigmented colloid milium from hydroquinone bleaching creams, 613

Foreman, M.I. and Clanachan, I., the percutaneous penetration of nandrolone decanoate, 47

Forman, L., pigmentation of the palms and scalp probably due to proprietary hair tonics, containing various phenols and phenolic derivatives, 718

Frain-Bell, W., Zaynoun, S., Gardiner, J.M.; Johnson, B.E. and: study of persistent light reaction in quindoxin and quinine photosensitivity (summary), Suppl. 11, 21

Frederici, H.H.R., Schmid, F.R., Kwaan, H.C.; Furey, Nancy L. and: arterial thrombosis in scleroderma, 683

From, E., methotrexate pneumonitis in a psoriatic, 107

Furey, Nancy L., Schmid, F.R., Kwaan, H.C. and Frederici, H.H.R., arterial thrombosis in scleroderma, 683

Gairdner, D., Rook, A.; Goldin, D. and: granuloma annulare in Mauriac's syndrome (case report), Suppl. 11, 31

Gajewska, M., rosacea of common male baldness, 63

Gange, R.W., De Bats, A., Park, J.R., Bradstreet, C.M.P. and Rhodes, E.L., cellular immunity and circulating antibody to herpes simplex virus in subjects with recurrent herpes simplex lesions and controls as measured by the mixed leukocyte migration inhibition test and complement fixation, 539

Gange, R.W., De Bats, A., Park, J.R., Bradstreet, C.M.P. and Rhodes, E.L., immunity in herpes simplex (summary), Suppl. 11, 15

Gardiner, J.M., Zaynoun, S., Frain-Bell, W.; Johnson, B.E. and: study of persistent light reaction in quindoxin and quinine photosensitivity (summary), Suppl. 11, 21

Garretts, M.; Pace, J.L. and: urticaria and hyperthyroidism, 97

GAYLARDE, P.M., BROCK, A.P., SARKANY, I.; BARNES, H. and: an improved method of assessing topical corticosteroid activity, 459

GENTLES, J.C., JONES, G.R. and ROBERTS, D.T., efficacy of miconazole in the topical treatment of tinea pedis in sportsmen, 79

GLUCKMAN, ELIANE, BUSSEL, ANETTE, DIDIERJEAN, LILIANE, PUISSANT, A.; SAURAT, J.H. and: the lichen planus-like eruption after bone marrow transplantation, 675

GOLDIN, D. and CHAMPION, R.H., follicular keratoses (case report), Suppl. 11, 48

GOLDIN, D. and CHAMPION, R.H., morphoea arising in a hypoplastic breast (case report), Suppl. 11, 44

GOLDIN, D., ROBERTS, S.O.B. and EVANS, D.B., lipid deposition in the dermis following renal transplant (case report), Suppl. 11, 59

GOLDIN, D. and ROOK, A., mycosis fungoides with necrotising angiitis (case report), Suppl. 11, 62

GOLDIN, D. and ROOK, A., pyoderma gangrenosum (case report), Suppl. 11, 31

GOLDIN, D., ROOK, A. and GAIRDNER, D., granuloma annulare in Mauriac's syndrome (case report), Suppl. 11, 31

GOLDIN, D., ROOK, A. and LAMB, W.T., plasma cell gingivitis (case report), Suppl. 11, 66

GOLDNER, R., CALTON, G.J.; BURNETT, J.W. and: Cowden disease, 329

GRANT, P.W. and McCALLUM, D.I., cutaneous aspects of mongolism—a clinical study (summary), Suppl. 11, 14

GRIFFITHS, W.A.D. and DANESHBOD, K., pseudoxanthomatous mastocytosis, 91

GRIFFITHS, W.A.D. and DUTZ, W., repeated tissue sampling with a dental broach. A trial in cutaneous leishmaniasis, 43

GUERRA RODRIGO, F., BITTENCOURT-SAMPAIO, S.; COTTA-PEREIRA, G. and: ultrastructural study of elaunin fibres in the secretory coil of human eccrine sweat glands, 623

GUERRA RODRIGO, F., COTTA-PEREIRA, G. and DAVID-FERREIRA, J.F., the fine structure of the elastic tendons in the human arrector pili muscle, 631

HALME, H., KIISTALA, U., NIEMI, K.-M., LASSUS, A.; JOHANSSON, E.A. and: the deoxyribonucleic acid (DNA) skin test in systemic lupus erythematosus. Clinical evaluation, 443; histological findings, 451

HALPRIN, K.M., ADACHI, K., LEVINE, V.; YOSHIKAWA, K. and: is the cyclic AMP in psoriatic epidermis low?, 253

HALPRIN, K.M., ADACHI, K., LEVINE, V.; YOSHIKAWA, K. and: the effects of catecholamine and related compounds on the adenyl cyclase system in the epidermis, 29

HAMERLINCK, F., CORMANE, R.H.; HUSZ, S. and: prealbumin bearing lymphocytes as an indicator of hapten protein carrier in certain varieties of dermatitis, 11

HAMMAR, H., studies on the energy metabolism in lichen planus, 667

HARTDEGEN, R.G., DOGLIOTTI, M., RABINOWITZ, L. and BARTLETT, R.G., leprechaunism, 587

HAYES, J.P., BYROM, N.A.; TAN, R.S.-H. and: a method of liberating living cells from the dermal infiltrate, 271

HAYES, J.P., BYRON, N.A.; TAN, R.S.-H. and: identification of lymphoid cells in the cutaneous infiltrate of skin reticuloses and lichen planus (summary), Suppl. 11, 16

HERRERO, C., ALMEIDA, J., SMITH, S.G., BELCHER, R.V.; PIÑOL AGUADÉ, J. and: thin layer chromatography and counter-current analysis in porphyrias, 277

HEWITT, J., BENVENISTE, M. and LESSANA-LEIBOWITCH, M., pemphigus induced by D-penicillamine (summary), Suppl. 11, 12

HEYL, T. and MORRISON, J.G.L., keratoacanthoma in a Bantu patient, 699

HOLLAND, K.T., CUNLIFFE, W.J.; ROBERTS, C.D. and: lipase, hyaluronidase and protease production from bacteria isolated from acne patients (summary), Suppl. 11, 26

HOLST, R. and MÖLLER, H., one hundred twin pairs patch tested with primary irritants, 145

HOLT, P. and MARKS, R., epidermal structure and metabolic activity in acromegaly (summary), Suppl. 11, 29

HOLTI, G., BURTON, J.L., WRIGHT, V.; CUNLIFFE, W.J. and: hazards of steroid therapy in hepatic failure, 183

HOLUBAR, K.; STINGL, G. and: coexistence of lichen planus and bullous pemphigoid, 313

HOPPER, F.E.; ROWELL, N.R. and: periodontal membrane in systemic sclerosis (summary), Suppl. 11, 23

HUNTER, J.A.A., PARKER, SHEENA, THOMPSON, G.G., MOORE, M.R.; ALLEN, B.R. and: effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria, 37

HURD, JANICE; ROBINSON, T. and: reactivation of *Herpesvirus hominis* (HVH) infection in mice treated with cyclophosphamide and prednisolone sodium phosphate (Codelsol) (summary), Suppl. 11, 24

HUSZ, S., HAMERLINCK, F. and CORMANE, R.H., prealbumin bearing lymphocytes as an indicator of hapten protein carrier in certain varieties of dermatitis, 11

HUTCHINSON, F., MACLEOD, T.M. and RAFFLE, E.J., nickel hypersensitivity, 557

JACYK, W.K., erythrocyte glucose-6-phosphate dehydrogenase in lichen planus (letter), 353

JOHANSSON, E.A., KIISTALA, U., NIEMI, K.-M., HALME, H. and LASSUS, A., the deoxyribonucleic acid (DNA) skin test in systemic lupus erythematosus. Clinical evaluation, 443; histological findings, 451

JOHNSON, B.E., ZAYNOUN, S., GARDINER, J.M. and FRAIN-BELL, W., study of persistent light reaction in quindoxin and quinine photosensitivity (summary), Suppl. 11, 21

Jones, G.R., Roberts, D.T.; Gentles, J.C. and: efficacy of miconazole in the topical treatment of tinea pedis in sportsmen, 79

Jones, H.E., Linamen, C.E., Akers, W.A.; Spencer, T.S. and: temperature dependence of water content of stratum corneum, 159

Kaminsky, C.A., Kaminsky, A.R.D., Sanguinetti, O. and Shaw, M., acquired pigmentation of skin folds with the histological picture of seborrhoeic wart, 713

Katz, S.A., Bowen, H.J.M., Comaish, J.S. and Samitz, M.H., tissue nickel levels and nickel dermatitis. I. Nickel in hair, 187

Kiistala, U., Niemi, K.-M., Halme, H., Lassus, A.; Johansson, E.A. and: the deoxyribonucleic acid (DNA) skin test in systemic lupus erythematosus. Clinical evaluation, 443; histological findings, 451

Kinmont, P.D.C., Wynn Williams, D., Cotton, R.E., Wroughton, M.A.; McCallum, D.I. and: intra-epidermal carcinoma of the eyelid margin, 239

Knight, A.G., Delaney, T.J., and Black, M.M., eosinophilic spongiosis; a clinical, histological and immunofluorescent correlation (summary), Suppl. 11, 13

Knight, A. and Vickers, C.F.H., a follow-up of tetracycline-treated rosacea with special reference to rosacea keratitis (summary), Suppl. 11, 12; 577

Knox, J.M., Duncan, W.C.; McBride, M.E. and: physiological and environmental control of Gram negative bacteria on skin, 191

Krivo, J.M.; Selmanowitz, V.J. and: pigmentary demarcation lines. Comparison of Negroes with Japanese, 371

Kubba, R. and Champion, R.H., anaphylactoid purpura caused by tartrazine and benzoates (case report), Suppl. 11, 61

Kubba, R. and Champion, R.H., calcinosis universalis (case report), Suppl. 11, 45

Kubba, R. and Champion, R.H., nickel sensitivity resembling bullous pemphigoid (case report), Suppl. 11, 41

Kubba, R. and Champion, R.H., scleromyxoedema (case report), Suppl. 11, 36

Kubba, R., McNeil, N.I. and Rook, A., lichen myxoedematosus, Crohn's disease and mucinous ovarian carcinoma (case report), Suppl. 11, 34

Kubba, R., Mitchell, J.N.S. and Rook, A., keratosis pilaris with recurrent folliculitis decalvans (case report), Suppl. 11, 55

Kubba, R. and Rook, A., Graham-Little syndrome. Follicular keratosis with cicatricial alopecia (case report), Suppl. 11, 53

Kwaan, H.C., Schmid, F.R., Frederici, H.H.R.; Furey, Nancy L. and: arterial thrombosis in scleroderma, 683

Lachapelle, J.M., Pierard, G.E. and Fierens, F., trichotillomania: a pathogenic interpretation of histologic changes in the pilosebaceous unit (summary), Suppl. 11, 21

Lamb, W.T., Rook, A.; Goldin, D. and: plasma cell gingivitis (case report), Suppl. 11, 66

Landells, J.W., Levene, G.M., Baker, H.; Warin, A.P. and: a prospective study of the effects of weekly oral methotrexate on liver biopsy, 321

Lang, W., Craig, J.A.; Thomson, J. and: deafness complicating mucous membrane pemphigoid, 337

Lassus, A., Kiistala, U., Niemi, K.-M., Halme, H.; Johansson, E.A. and: the deoxyribonucleic acid (DNA) skin test in systemic lupus erythematosus. Clinical evaluation, 443; histological findings, 451

Lehmuskallio, E.A., hair fragment in the skin resembling larva migrans, 349

Leppard, Barbara, Thomson, J.; Noble, W.C. and: the bacteriology of skin cysts (summary), Suppl. 11, 25

Lessana-Leibowitch, M., Benveniste, M.; Hewitt, J. and: pemphigus induced by D-penicillamine (summary), Suppl. 11, 12

Levantine, A. and Brostoff, J., immunological responses of patients with psoriasis and the effect of treatment with methotrexate, 659

Levene, G.M., Landells, J.W., Baker, H.; Warin, A.P. and: a prospective study of the effects of weekly oral methotrexate on liver biopsy, 321

Levine, V., Adachi, K., Halprin, K.M.; Yoshikawa, K. and: is the cyclic AMP in psoriatic epidermis low?, 253

Levine, V., Adachi, K., Halprin, K.M.; Yoshikawa, K. and: the effects of catecholamine and related compounds on the adenyl cyclase system in the epidermis, 29

Liddell, K., acne and eczema—a familial study (summary), Suppl. 11, 10

Linamen, C.E., Akers, W.A., Jones, H.E.; Spencer, T.S. and: temperature dependence of water content of stratum corneum, 159

Lowe, N.J., Cudworth, A.G. and Vickers, C.F.H., lichen planus: investigations of carbohydrate metabolism and the HLA system (summary), Suppl. 11, 18

Lyon, J.B., epithelioid sarcoma of the pulp of the left ring finger (case report), Suppl. 11, 71

Lyon, J.B., juvenile generalized pustular psoriasis (case report), Suppl. 11, 40

Lyon, J.B., pili torti (case report), Suppl. 11, 64

Lyon, J.B., xanthoma tuberosum and gout (case report), Suppl. 11, 57

McBride, M.E., Duncan, W.C. and Knox, J.M., physiological and environmental control of Gram negative bacteria on skin, 191

McCallum, D.I.; Grant, P.W. and: cutaneous aspects of mongolism—a clinical study (summary) Suppl. 11, 14

McCallum, D.I., Kinmont, P.D.C., Wynn Williams, D., Cotton, R.E. and Wroughton, M.A., intra-epidermal carcinoma of the eyelid margin, 239

Mackey, J.P., necrobiosis lipoidica diabeticorum involving scalp and face (case), 729

Macleod, T.M., Raffle, E.J.; Hutchinson, F. and: nickel hypersensitivity, 557

McNeil, N.I., Rook, A.; Kubba, R. and: lichen myxoedematosus, Crohn's disease and mucinous ovarian carcinoma (case report), Suppl. 11, 34

McVitie, Eva, Black, M.M.; Shuster, S. and: the influence of age and sex on skin thickness, skin collagen and density, 639

Mahrle, G. and Organos, C.E., ultrastructural localization and differentiation of membrane-bound ATP utilizing enzymes including adenyl cyclase in normal and psoriatic epidermis, 495

Main, R.A., Marsden, R.A.; Adams, Rowan H. and: a controlled study of vitamin E treatment in epidermolysis bullosa (summary), Suppl. 11, 10

Mali, J.W.H., alopecia areata (letter), 605

Marien, K.J.C. and Smeenk, G., plane xanthomata associated with multiple myeloma and hyperlipoproteinaemia, 407

Marks, R., increased DNA and protein synthesis in the involved and uninvolved skin of patients with psoriasis (summary), Suppl. 11, 28

Marks, R.; Bentley-Phillips, C.B. and: are moles just naevus cells? (summary), Suppl. 11, 15

Marks, R.; Holt, P. and: epidermal structure and metabolic activity in acromegaly (summary), Suppl. 11, 29

Marks, R., Pongsehirun, D.; Wilson Jones, E. and: naevus superficialis lipomatosis, 121

Marks, R., Presbury, D.G.C., Ramnarain, D.; Abell, E. and: the diagnostic significance of immunoglobulin and fibrin deposition in lichen planus, 17

Marks, R., Wilson Jones, E.; Abell, E. and: secondary syphilis: a clinico-pathological review, 53

Marriott, P.J., Munro, D.D. and Ryan, T., angioma serpiginosum—familial incidence, 701

Mars, P.W., Rabson, A.R., Rippey, J.J.; Dogliotti, M. and: cutaneous protothecosis (letter), 473

Marsden, R.A., Main, R.A.; Adams, Rowan H. and: a controlled study of vitamin E treatment in epidermolysis bullosa (summary), Suppl. 11, 10

Mauro, Jacqueline, Rayhanzadeh, S., Rudolsky, U.H.; Dantzig, P.I. and: the significance of a positive cutaneous immunofluorescence test in systemic lupus erythematosus, 531

Mier, P.D. and Van Den Hurk, J.J.M.A., inhibition of lysosomal enzymes by dapsone, 471

Mier, P.D. and Van Den Hurk, J.J.M.A., lysosomal hydrolases of the epidermis. 1. Glycosidases, 1. 2. Ester hydrolases, 391. 3. Peptide hydrolases, 509

Mier, P.D. and Van Den Hurk, J.J.M.A., membrane-bound ATP-hydrolytic activity of psoriatic epidermis, 469

Milne, J.A., the choice and use of equipment for photomicrographs, 231

Minocha, Y., Sood, V.D.; Pasricha, J.S. and: treatment of pemphigus with cyclophosphamide, 573

Mischler, T.W., Neiss, E.S., Shaw, J.M., Schreiber, E.C.; Dreyfuss, J. and: absorption and biotransformation of topically applied 8-(methylthio) cyclic AMP, 379

Mitchell, J.N.S., Rook, A.; Kubba, R. and: keratosis pilaris with recurrent folliculitis decalvans (case report), Suppl. 11, 55

Møller, H.; Holst, R. and: one hundred twin pairs patch tested with primary irritants, 145

Moore, M.R., Parker, Sheena, Thompson, G.G., Darby, F.J., Hunter, J.A.A.; Allen, B.R. and: effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria, 37

Morison, W.L., cell-mediated immune responses in patients with warts, 553

Morison, W.L., *in vitro* assay of immunity to human wart antigen, 545

Morison, W.L., wart immunity, autoantibodies and Australia antigen in sarcoidosis, 717

Morison, W.L., Rhodes, Ellen L.; Bye, P.G.T. and: the absorption of hexachlorophane from Ultralanum ointment, 209

Morris, T.C.M., Bridges, J.M.; Burrows, D. and: reactivation of scabies rash by methotrexate, 219

Morrison, J.G.L.; Heyl, T. and: keratoacanthoma in a Bantu patient, 699

Morrison, J.G.L., Simson, I.W.; Findlay, G.H. and: exogenous ochronosis and pigmented colloid milium from hydroquinone bleaching creams, 613

Moschella, S.L., hypersensitive and insensitive extremities. The dermatological aspects, 151

Munro, D.D., Ryan, T.; Marriott, P.J. and: angioma serpiginosum—familial incidence, 701

Naylor, P.F.D., Sayers, B.McA.; Evans, N.T.S. and: fluctuations in the blood flow of forearm skin (summary), Suppl. 11, 25

Neering, H., vitiligo and jaundice, 229

Neiss, E.S., Mischler, T.W., Shaw, J.M., Schreiber, E.C.; Dreyfuss, J. and: absorption and biotransformation of topically applied 8-(methylthio) cyclic AMP, 379

Nicholls, Anne; Champion, R.H. and: relapsing polychondritis (case report), Suppl. 11, 67

Niemi, K.-M., Kiistala, U., Halme, H., Lassus, A.; Johansson, E.A. and: the deoxyribonucleic acid (DNA) skin test in systemic lupus erythematosus. Clinical evaluation, 443; histological findings, 451

Noble, W.C., dispersal of skin micro-organisms (review), 477

Noble, W.C., Leppard, Barbara, and Thomson, J., the bacteriology of skin cysts (summary), Suppl. 11, 25

Noble, W.C.; Savin, J.A. and: immunosuppression and skin infection, 115

Odland, G.F.; Eady, R.A.J. and: intraendothelial tubular aggregates in experimental wounds, 165

Opdyke, D.L., the safety of fragrance ingredients, 351

Orfanos, C.E.; Mahrle, G. and: ultrastructural localization and differentiation of membrane-bound ATP utilizing enzymes including adenyl cyclase in normal and psoriatic epidermis, 495

Oswald, I., Paterson, W.D., Adam, Kirstine; Savin, J.A. and: further studies of scratching during sleep, 297

Pace, J.L. and Garretts, M., urticaria and hyperthyroidism, 97

Parish, W.E., Champion, R.H.; Welbourn, Esther, and: nummular eczema with staphylococcal sensitivity (case report), Suppl. 11, 40

Park, J.R., De Bats, A., Bradstreet, C.M.P., Rhodes, E.L.; Gange, R.W. and: cellular immunity and circulating antibody to herpes simplex virus in subjects with recurrent herpes simplex lesions and controls as measured by the mixed leukocyte migration inhibition test and complement fixation, 539

Park, J.R., De Bats, A., Bradstreet, C.M.P., Rhodes, E.E.; Gange, R.W. and: immunity in herpes simplex (summary), Suppl. 11, 15

Parker, Sheena, Thompson, G.G., Moore, M.R., Darby, F.J., Hunter, J.A.A.; Allen, B.R. and: effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria, 37

Pasricha, J.S., Sood, V.D. and Minocha, Y., treatment of pemphigus with cyclophosphamide, 573

Paterson, W.D., Oswald, I., Adam, Kirstine; Savin, J.A. and: further studies of scratching during sleep, 297

Pegum, J.S., induced tolerance in physical urticarias (letter), 473

Perera, W.D.H., Williams, Margaret, Williams, Susan, Tan, Giaw; Cunliffe, W.J. and: keratin hydration and sebum excretion rate (summary), Suppl. 11, 27

Pierard, G.E., Fierens, F.; Lachapelle, J.M. and: trichotillomania: a pathogenic interpretation of histologic changes in the pilosebaceous unit (summary), Suppl. 11, 21

Piñol Aguadé, J., Herrero, C., Almeida, J., Smith, S.G. and Belcher, R.V., thin layer chromatography and counter-current analysis in porphyrias, 277

Pollitt, N.T., β-carotene and the photodermatoses, 721

Pongsehirun, D., Marks, R.; Wilson Jones, E. and: naevus superficialis lipomatosis, 121

Potten, C.S., epidermal transit times, 649

Presbury, D.G.C., Marks, R., Ramnarain, D.; Abell, E. and: the diagnostic significance of immunoglobulin and fibrin deposition in lichen planus, 17

Puissant, A., Gluckman, Eliane, Bussel, Anette, Didierjean, Liliane; Saurat, J.H. and: the lichen planus-like eruption after bone marrow transplantation, 675

Rabinowitz, L., Dogliotti, M., Bartlett, R.G.; Hartdegen, R.G. and: leprechaunism, 587

Rabson, A.R., Mars, P.W., Rippey, J.J.; Dogliotti, M. and: cutaneous protothecosis (letter), 473

Raffle, E.J., Macleod, T.M.; Hutchinson, F. and: nickel hypersensitivity, 557

Ramnarain, D., Presbury, D.G.C., Marks, R.; Abell, E. and: the diagnostic significance of immunoglobulin and fibrin deposition in lichen planus, 17

Rayhanzadeh, S., Mauro, Jacqueline, Rudofsky, U.H.; Dantzig, P.I. and: the significance of a positive cutaneous immunofluorescence test in systemic lupus erythematosus, 531

Rhodes, E.L., De Bats, A., Park, J.R., Bradstreet, C.M.P.; Gange, R.W. and: cellular immunity and circulating antibody to herpes simplex virus in subjects with recurrent herpes simplex lesions and controls as measured by the mixed leukocyte migration inhibition test and complement fixation, 539

Rhodes, E.L., De Bats, A., Park, J.R., Bradstreet, C.M.P.; Gange, R.W. and: immunity in herpes simplex (summary), Suppl. 11, 15

Rhodes, E.L., Morison, W.; Bye, P.G.T. and: the absorption of hexachlorophane from Ultralanum ointment, 209

Rippey, J.J., Mars, P.W., Rabson, A.R.; Dogliotti, M. and: cutaneous protothecosis (letter), 473

Roberts, C.D., Holland, K.T. and Cunliffe, W.J., lipase, hyaluronidase and protease production from bacteria isolated from acne patients (summary), Suppl. 11, 26

Roberts, D.T., Jones, G.R.; Gentles, J.C. and: efficacy of miconazole in the topical treatment of tinea pedis in sportsmen, 79

Roberts, S.O.B., Evans, D.B.; Goldin, D. and: lipid deposition in the dermis following renal transplant (case report), Suppl. 11, 59

Robinson, T. and Hurd, Janice, reactivation of *Herpesvirus hominis* (HVH) infection in mice treated with cyclophosphamide and prednisolone sodium phosphate (Codelsol) (summary), Suppl. 11, 24

Rook, A., Gairdner, D.; Goldin, D. and: granuloma annulare in Mauriac's syndrome (case report), Suppl. 11, 31

Rook, A.; Goldin, D. and: mycosis fungoides with necrotising angiitis (case report), Suppl. 11, 62

Rook, A.; Goldin, D. and: pyoderma gangrenosum (case report), Suppl. 11 31

Rook, A.; Kubba, R. and: Graham–Little syndrome. Follicular keratosis with cicatricial alopecia (case report), Suppl. 11, 53

Rook, A., Lamb, W.T.; Goldin, D. and: plasma cell gingivitis (case report), Suppl. 11, 66

Rook, A., McNeil, N.I.; Kubba, R. and: lichen myxoedematosus, Crohn's disease and mucinous ovarian carcinoma (case report), Suppl. 11, 34

Rook, A., Mitchell, J.N.S.; Kubba, R. and: keratosis pilaris with recurrent folliculitis decalvans (case report), Suppl. 11, 55

Rowell, N.R. and Hopper, F.E., periodontal membrane in systemic sclerosis (summary), Suppl. 11, 23

Rowell, N.R. and Scott, D.G., immunohistological studies, with anti-connective tissue and anti-immunoglobulin antisera, of the skin in lupus erythematosus and scleroderma, 431

Rudofsky, U.H., Mauro, Jacqueline, Rayhanzadeh, S.; Dantzig, P.I. and: the significance of a positive cutaneous immunofluorescence test in systemic lupus erythematosus, 531

Ryan, T.J., erythropoietic protoporphyrias: submicroscopic events during the acute photosensitivity flare (letter), 605
Ryan, T.J.; Coles, R.B. and: the psoriasis sufferer in the community, 111
Ryan, T.J., Munro, D.D.; Marriott, P.J. and: angioma serpiginosum—familial incidence, 701

Samitz, M.H., Bowen, H.J.M., Comaish, J.S.; Katz, S.A. and: tissue nickel levels and nickel dermatitis. I. Nickel in hair, 187
Sanguinetti, O., Kaminsky, A.R.D., Shaw, M.; Kaminsky, C.A. and: acquired pigmentation of skin folds with the histological picture of seborrhoeic wart, 713
Sarkany, I., Gaylarde, P.M., Brock, A.P.; Barnes, H. and: an improved method of assessing topical corticosteroid activity, 459
Saurat, J.H., Gluckman, Eliane, Bussel, Anette, Didierjean, Liliane, and Puissant, A., the lichen planus-like eruption after bone marrow transplantation, 675
Savin, J.A., international mortality from bullous diseases since 1950 (summary), Suppl. 11, 17
Savin, J.A. and Noble, W.C., immunosuppression and skin infection, 115
Savin, J.A., Paterson, W.D., Oswald, I. and Adam, Kirstine: further studies of scratching during sleep, 297
Sayers, B.McA., Naylor, P.F.D.; Evans, N.T.S. and: fluctuations in the blood flow of forearm skin (summary), Suppl. 11, 25
Schmid, F.R., Kwaan, H.C., Frederici, H.H.R.; Furey, Nancy L. and: arterial thrombosis in scleroderma, 683
Schreiber, E.C., Mischler, T.W., Neiss, E.S., Shaw, J.M.; Dreyfuss, J. and: absorption and biotransformation of topically applied 8-(methylthio)cyclic AMP, 379
Schultz, H., human infestation by *Ophionyssus natricis* snake mite, 695
Schutt, W.H., Caldwell, I.W.; Burton, J.L. and: hypertrichosis due to diazoxide, 707
Scott, D.G.; Rowell, N.R. and: immunohistological studies, with anti-connective tissue and anti-immunoglobulin antisera, of the skin in lupus erythematosus and scleroderma, 431
Selmanowitz, V.J. and Krivo, J.M., pigmentary demarcation lines. Comparison of Negroes with Japanese, 371
Selwyn, S., natural antibiosis among skin bacteria as a primary defence against infection, 487
Serri, F.; Di Silverio, A. and: generalized bullous and haemorrhagic lichen sclerosus et atrophicus, 215
Seville, R.H., simplified dithranol treatment for psoriasis, 205
Shaw, J.M., Mischler, T.W., Neiss, E.S., Schreiber, E.C.; Dreyfuss, J. and: absorption and biotransformation of topically applied 8-(methylthio)cyclic AMP, 379
Shaw, M., Kaminsky, A.R.D., Sanguinetti, O.; Kaminsky, C.A. and: acquired pigmentation of skin folds with the histological picture of seborrhoeic wart, 713
Shuster, S., Black, M.M. and McVitie, Eva: the influence of age and sex on skin thickness, skin collagen and density, 639
Shuster, S.; Felix, R. and: a new method for the measurement of itch and the response to treatment, 303
Simson, I.W., Morrison, J.G.L.; Findlay, G.H. and: exogenous ochronosis and pigmented colloid milium from hydroquinone bleaching creams, 613
Singh, J. and Beck, G.A., folliculitis (case report), Suppl. 11, 49
Singh, J. and Beck, G.A., morphoea following chicken pox (case report), Suppl. 11, 43
Smeenk, G.; Marien, K.J.C. and: plane xanthomata associated with multiple myeloma and hyperlipoproteinaemia, 407
Smith, M.A., discoid lupus erythematosus (case report), Suppl. 11, 38
Smith, M.A., sarcoidosis (case report), Suppl. 11, 68
Smith, Ruth J.; Warin, R.P. and: challenge test battery in chronic urticaria (summary), Suppl. 11, 19
Smith, S.G., the use of thin layer chromatography in the separation of free porphyrins and porphyrin methyl esters, 291
Smith, S.G., Herrero, C., Almeida, J., Belcher, R.V.; Piñol Aguadé, J. and: thin layer chromatography and counter-current analysis in porphyrias, 277
Sood, V.D., Minocha, Y.; Pasricha, J.S. and: treatment of pemphigus with cyclophosphamide, 573
Sparrow, G. and Abell, E., granuloma annulare and necrobiosis lipoidica treated by jet injector, 85
Spencer, T.S., Linamen, C.E., Akers, W.A. and Jones, H.E., temperature dependence of water content of stratum corneum, 159
Stankler, L., deficiency of circulating IgA and IgM in adult patients with lichen planus, 25
Stankler, L. and Ewen, S.W.B., Hodgkin's disease in a patient with follicular and dermal mucinosis and spontaneous ooze of mucin (mucinorrhoea), 581
Stingl, G. and Holubar, K., coexistence of lichen planus and bullous pemphigoid, 313
Stoughton, R.B.; Du Vivier, A. and: an animal model for screening topical and systemic drugs for potential use in the treatment of psoriasis using a new, rapid and simple technique of extracting DNA from epidermis involving hydroxyapatite column chromatography (summary), Suppl. 11, 27
Sutherst, J.R.; Ward, G.D. and: pruritus vulvae: treatment by multiple intradermal alcohol injections, 201

Tan, Giaw, Perera, W.D.H., Williams, Margaret, Williams, Susan; Cunliffe, W.J. and: keratin hydration and sebum excretion rate (summary), Suppl. 11, 27

Tan, R.S.-H., Byrom, N.A. and Hayes, J.P. a method of liberating living cells from the dermal infiltrate, 271

Tan, R.S.-H., Byrom, N.A. and Hayes, J.P., identification of lymphoid cells in the cutaneous infiltrate of skin reticuloses and lichen planus (summary), Suppl. 11, 16

Tan, S.G. and Cunliffe, W.J., high doses of ascorbic acid (letter), 731

Tan, S.G., Tebbs, E.; Cunliffe, W.J. and: rosacea, migraine, Dixarit (summary), Suppl. 11, 11

Tan, S.G., Williams, S.M.; Cunliffe, W.J. and: sebum excretion rate investigations: a new absorbent paper, 347

Tebbs, E., Tan, S.G.; Cunliffe, W.J., and: rosacea, migraine, Dixarit (summary), Suppl. 11, 11

Thiele, F.A.J., De Jongh, G.J.; Cotton, D.W.K. and: Fourier analysis of thermal sweating, 223

Thivolet, J.; Claudy, A. and: multiple keratoacanthomas: association with deficient cell mediated immunity, 593

Thompson, G.G., Parker, Sheena, Moore, M.R., Darby, F.J., Hunter, J.A.A.; Allen, B.R. and: effect of treatment on plasma uroporphyrin levels in cutaneous hepatic porphyria, 37

Thomson, J. Lang, W. and Craig, J.A., deafness complicating mucous membrane pemphigoid, 337

Thomson, J., Leppard, Barbara; Noble, W.C. and: the bacteriology of skin cysts (summary), Suppl. 11, 25

Van Den Hurk, J.J.M.A.; Mier, P.D. and: inhibition of lysosomal enzymes by dapsone, 471

Van Den Hurk, J.J.M.A.; Mier, P.D. and: lysosomal hydrolases of the epidermis. 1. Glycosidases, 1. 2. Ester hydrolases, 391. 3. Peptide hydrolases, 509

Van Den Hurk, J.J.M.A.; Mier, P.D. and: membrane-bound ATP-hydrolytic activity of psoriatic epidermis, 469

Van Der Staak, W.B.J.M.; Cotton, D.W.K. and: erythrocyte glucose-6-phosphate dehydrogenase in lichen planus (letter), 354

Van Hasselt, P., Bergers, A.M.S.; Cotton, D.W.K. and: electron microscopy of the sweat glands from the hairy skin of the beagle, 348

Verbov, J., palmar ridge appearances in normal newborn infants and ridge appearance in relation to eccrine sweating, 645

Vickers, C.F.H., Cudworth, A.G.; Lowe, N.J. and: lichen planus: investigations of carbohydrate metabolism and the HLA system (summary), Suppl. 11, 18

Vickers, C.F.H.; Knight, A. and: a follow-up of tetracycline-treated rosacea with special reference to rosacea keratitis (summary), Suppl. 11, 12; 577

Walker, Anne E., preliminary report of a vascular abnormality occurring in men engaged in the manufacture of polyvinyl chloride (summary), Suppl. 11, 22

Ward, G.D. and Sutherst, J.R., pruritus vulvae: treatment by multiple intradermal alcohol injections, 201

Warin, A.P., Landells, J.W., Levene, G.M. and Baker, H., a prospective study of the effects of weekly oral methotrexate on liver biopsy, 321

Warin, R.P. and Smith, Ruth J., challenge test battery in chronic urticaria (summary), Suppl. 11, 19

Welbourn, Esther, Champion, R.H. and Parish, W.E., nummular eczema with staphylococcal sensitivity (case report), Suppl. 11, 40

Williams, Margaret, Perera, W.D.H., Williams, Susan, Tan, Giaw; Cunliffe, W.J. and: keratin hydration and sebum excretion rate (summary), Suppl. 11, 27

Williams, S.M., Tan, S.G.; Cunliffe, W.J. and: sebum excretion rate investigations, 347

Williams, S.M., Perera, W.D.H., Williams, Margaret, Tan, Giaw; Cunliffe, W.J. and: keratin hydration and sebum excretion rate (summary), Suppl. 11, 27

Wilson Jones, E., Marks, R.; Abell, E. and: secondary syphilis: a clinico-pathological review, 53

Wilson Jones, E., Marks, R. and Pongsehirun, D., naevus superficialis lipomatosis, 121

Woodford, R.; Barry, B.W. and: comparative bioavailability and activity of proprietary topical corticosteroid preparations: vasoconstrictor assays on thirty-one ointments, 563

Wrench, Rosanne and Britten, A.Z., evaluation of coal tar fractions for use in psoriasiform diseases using the mouse tail test. III. High boiling tar oil acids, 67

Wrench, Rosanne and Britten, A.Z., evaluation of dithranol and a 'synthetic tar' as antipsoriatic treatments using the mouse tail test, 75

Wright, V., Burton, J.L., Holti, G.; Cunliffe, W.J. and: hazards of steroid therapy in hepatic failure, 183

Wroughton, M.A., Kinmont, P.D.C., Wynn Williams, D., Cotton, R.E.; McCallum, D.I. and: intra-epidermal carcinoma of the eyelid margin, 239

Wyatt, E.H., lichen planus and ulcerative colitis, 465

Wynn Williams, D., Kinmont, P.D.C., Cotton, R.E., Wroughton, M.A.; McCallum, D.I. and: intra-epidermal carcinoma of the eyelid margin, 239

Yoshikawa, K., Adachi, K., Halprin, K.M. and Levine, V., is the cyclic AMP in psoriatic epidermis low?, 253

Yoshikawa, K., Adachi, K., Halprin, K.M. and Levine, V., the effects of catecholamine and related compounds on the adenyl cyclase system in the epidermis, 29

Zaynoun, S., Gardiner, J.M., Frain-Bell, W.; Johnson, B.E. and: study of persistent light reaction in quindoxin and quinine photosensitivity (summary), Suppl. 11, 21

Dermatologica

Offizielles Organ der Schweizerischen Gesellschaft für Dermatologie und Venerologie
Organe officiel de la Société Suisse de Dermatologie et de Vénérologie
Official Organ of the Nederlandse Vereniging van Dermatologen

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Index Vol. 150, No. 4, 1975

Proceedings · Verhandlungsberichte · Congrès

56. Jahresversammlung der Schweizerischen Gesellschaft für Dermatologie und Venereologie, 18. und 19. Oktober 1974 in Lausanne – 56e Réunion annuelle de la Société Suisse de Dermatologie et de Vénéréologie, les 18 et 19 octobre 1974 à Lausanne

Mitteilungen · Communications

Brun, R. (Genève): Statistique des tests épicutanés positifs de 1000 cas d'eczéma de contact (Epidemiology of Contact Dermatitis in Geneva [1,000 Cases])

Bader, C.; Orusco, M. et Laugier, P. (Genève): Recherche systématique de la blennorragie dans une consultation de planning familial
(Value of Screening for Gonorrhea at a Family Planning Consultation)

Abstracts

Demonstrationen · Démonstrations

Rufli, Th. (Basel)

Laugier, P.; Hunziker, N.; Orusco, M.; Brun, R.; Reiffers, J. et Posternak, F. (Genève)

Delacrétaz, J.; Frenk, E. et Tapernoux, B. (Lausanne)

Kaufmann, J.; Gilliet, F.; Ott, F. und Wüthrich, B. (Zürich)

Krebs, A. und Mitarbeiter (Bern)

Pillai, K.G.; Singh, G., and Sharma, B.M. (Varanasi): *Trichophyton rubrum* Infection of the Penis

Subscription date: 'Dermatologica' appears monthly in numbers of approximately 64 pages each.
6 numbers form 1 volume and 2 volumes are issued annually.
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of Great Britain

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VOL. 27 NO. 1 JANUARY 1976

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'Manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 93, Number 6, December 1975

CONTENTS

Clinical and Laboratory Investigations

Page

Exogenous Ochronosis and Pigmented Colloid Milium from Hydroquinone Bleaching Creams 613
G. H. FINDLAY, J. G. L. MORRISON AND I. W. SIMSON

Ultrastructural Study of Elaunin Fibres in the Secretory Coil of Human Eccrine Sweat Glands 623
G. COTTA-PEREIRA, F. GUERRA RODRIGO AND S. BITTENCOURT-SAMPAIO

The Fine Structure of the Elastic Tendons in the Human *Arrector Pili* Muscle 631
F. GUERRA RODRIGO, G. COTTA-PEREIRA AND J. F. DAVID-FERREIRA

The Influence of Age and Sex on Skin Thickness, Skin Collagen and Density 639
SAM SHUSTER, MARTIN M. BLACK AND EVA MCVITIE

Palmar Ridge Appearances in Normal Newborn Infants and Ridge Appearance in Relation to Eccrine Sweating 645
JULIAN VERBOV

Epidermal Transit Times 649
CHRISTOPHER S. POTTEN

Immunological Responses of Patients with Psoriasis and the Effect of Treatment with Methotrexate 659
A. LEVANTINE AND JONATHAN BROSTOFF

Studies on the Energy Metabolism in Lichen Planus 667
HANS HAMMAR

The Lichen Planus-like Eruption after Bone Marrow Transplantation 675
J. H. SAURAT, ELIANE GLUCKMAN, ANETTE BUSSEL, LILIANE DIDIERJEAN AND A. PUISSANT

Arterial Thrombosis in Scleroderma 683
NANCY L. FUREY, F. R. SCHMID, H. C. KWAAN AND H. H. R. FRIEDERICI

Case Reports

Human Infestation by *Ophionyssus Natricis* Snake Mite 695
HANNE SCHULTZ

Keratoacanthoma in a Bantu Patient 699
T. HEYL AND J. G. L. MORRISON

Angioma Serpiginosum—Familial Incidence 701
PHILIP J. MARRIOTT, DOWLING D. MUNRO AND TERENCE RYAN

Hypertrichosis due to Diazoxide 707
J. L. BURTON, W. H. SCHUTT AND I. W. CALDWELL

Acquired Pigmentation of Skin Folds with the Histological Picture of Seborrhoeic Wart 713
C. A. KAMINSKY, A. R. DE KAMINSKY, O. SANGUINETTI AND M. SHAW

Brief Communications

Wart Immunity, Autoantibodies and Australia Antigen in Sarcoidosis 717
WARWICK L. MORISON

Pigmentation of the Palms and Scalp Probably due to Proprietary Hair Tonics, Containing Various Phenols and Phenolic Derivatives 718
L. FORMAN

Erythema Nodosum from *Yersinia Enterocolitica* 719
N. E. BALDOCK AND M. D. CATTERALL

Comment

β-Carotene and the Photodermatoses 721
NORMAN POLLITT

Obituaries

DR W. N. GOLDSMITH 725
DR F. GLYN-HUGHES 728

Society Proceedings

Irish Dermatological Society 729

Correspondence

High Doses of Ascorbic Acid 731
S. G. TAN AND W. J. CUNLIFFE

News and Notices 732

Index to Volume 93 733

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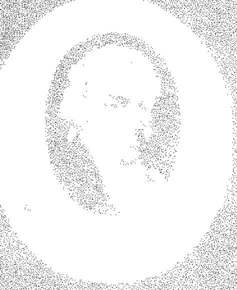
Volume 93, Supplement 11, July 1975
Annual Meeting Cambridge 1975



British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

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British Journal of Dermatology is published monthly at £1.75 ($6.00) per issue; subscription price £18.00 ($63.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed and bound in Great Britain at the Alden Press, Oxford

British Journal of Dermatology (1975) **93**, Supplement **11**, 1.

Foreword

As in 1974, this special supplement contains summaries of the papers and details of the cases to be presented at the fifty-fifth annual meeting of the British Association of Dermatologists. Those attending the meeting are asked to bring with them this supplement, which is also being distributed free to all subscribers to the journal thanks once again to the generosity of Glaxo Laboratories Limited.

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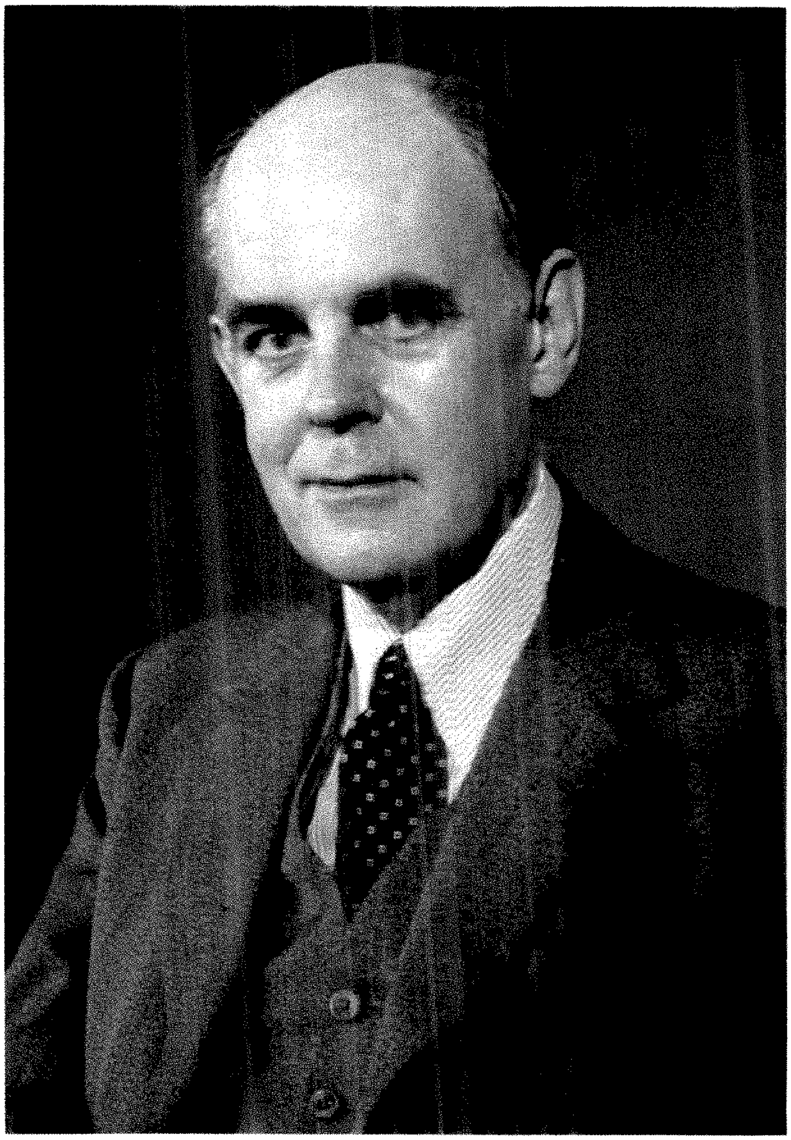

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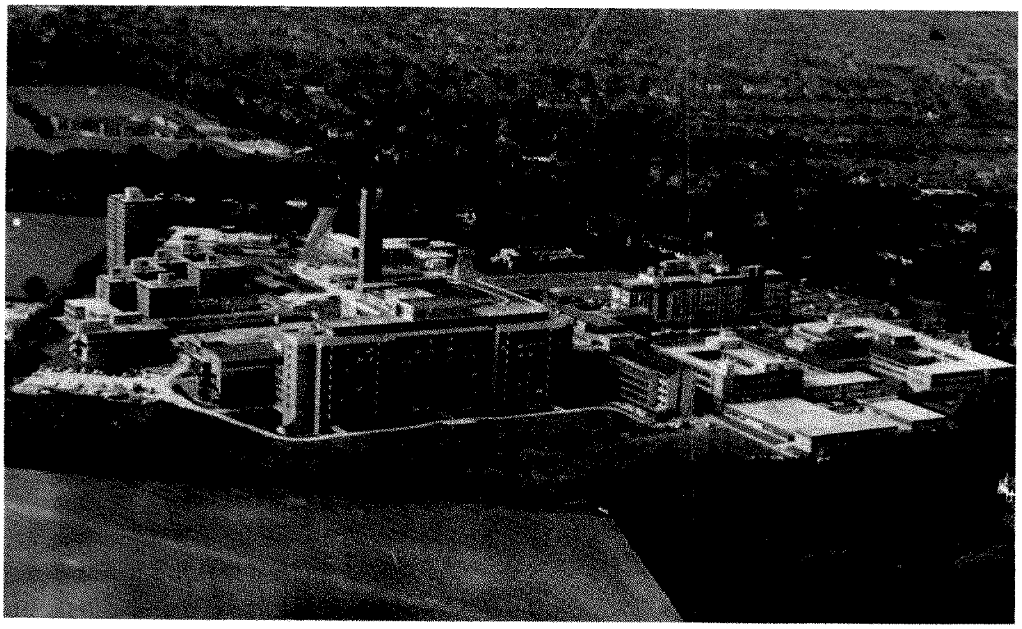

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British Journal of Dermatology (1975) 93, Supplement 11, 4.

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PRESIDENT: DR ARTHUR ROOK

PROGRAMME OF MEETING

Thursday 3 July

Lecture Theatre No. 1, The Chemical Laboratory, Lensfield Road, Cambridge

08.45	Registration	
09.15	Annual General Meeting (Members only)	
10.15	**Coffee**	
10.40	G.H.Findlay	Ochronosis and colloid milium from hydroquinone in man
11.00	Stanley S.Bleehen	The treatment of hypermelanosis with 4-isopropylcatechol
11.20	Rowan H.Adams, R.A.Main and R.A.Marsden	A controlled study of vitamin E treatment in epidermolysis bullosa
11.40	K.Liddell	Acne and eczema—a familial study
12.00	W.J.Cunliffe, S.G.Tan and E.Tebbs	Rosacea, migraine, dixarit
12.20	Arthur Knight and C.F.H.Vickers	A follow-up of tetracycline treated rosacea (with special reference to rosacea keratitis)
12.40	**Lunch**	

14.00	Jean Hewitt, M. Benveniste and M. Lessana-Leibowitch	Pemphigus induced by D. penicillamine
14.20	A.G.Knight, T.J.Delaney and M.M.Black	Eosinophilic spongiosis, a clinical, histological and immunofluorescent correlation
14.40	R.D.Azulay	Personal experience with clofazimine in the treatment of leprosy
15.00	P.W.Grant and D.I.McCallum	Cutaneous aspects of mongolism—a clinical study
15.20	R.W.Gange, C.M.P.Bradstreet, A.DeBats, J.R.Park and E.L.Rhodes	Immunity in herpes simplex
15.40	**Tea**	
16.10	C.B.Bentley-Phillips and R.Marks	Are moles just naevus cells?
16.30	E.A.J.Eady and T.C.Cowen	Naevus spilus: a light and electron microscopical study
16.50	R.S-H.Tan, J.P.Hayes and N.A.Byrom	Identification of lymphoid cells in the cutaneous infiltrate of skin reticuloses and lichen planus
18.30	President's Reception at Trinity College	

Friday 4 July

Lecture Theatre No. 1, The Chemical Laboratory, Lensfield Road, Cambridge

08.45	Registration	
09.10	J.A.Savin	International mortality from bullous diseases since 1950
09.30	J.A.Carruthers and M.M.Black	Immunopathologic findings in herpes gestationis
00.50	N.J.Lowe and A.G.Cudworth	Lichen planus: investigations of carbohydrate metabolism and the HLA system

10.10	Robert P.Warin and Ruth J.Smith	Challenge test battery in chronic urticaria
10.30	J.M.Lachapelle, G.E.Pierard and F.Fierens	Trichotillomania: a pathogenic interpretation of histologic changes in the pilosebaceous unit
10.50	**Coffee**	
11.20	B.E.Johnson, S.Zaynoun, J.M.Gardiner and W.Frain-Bell	A study of persistent light reaction in quindoxin and quinine photosensitivity
11.40	Anne E.Walker	A preliminary report of a vascular abnormality occurring in men engaged in the manufacture of polyvinyl chloride
12.00	N.R.Rowell and F.E.Hopper	The Periodontal Membrane in Systemic Sclerosis
12.20	Rodney P.R.Dawber and J.P.H.Byrne	Finger-nail Growth in Peripheral Pustulosis
12.40	**Lunch**	

Afternoon Programme of Papers in Association with the Investigative Group

14.00	Trevor Robinson and Janice Hurd	Reactivation of herpes virus hominis infection in mice treated with cyclophosphamide and prednisolone sodium phosphate (Codelsol)
14.20	N.T.S.Evans, P.F.D.Naylor and B.McA.Sayers	Fluctuations in the blood flow of forearm skin
14.40	W.C.Noble, Barbara Leppard and J.Thomson	The bacteriology of skin cysts
15.00	Christopher D.Roberts, K.T.Holland and W.J.Cunliffe	Lipase, hyaluronidase and protease production from bacteria isolated from acne patients
15.20	W.J.Cunliffe, W.D.H.Perera, Margaret Williams, Susan Williams and Giaw Tan	Keratin hydration and sebum excretion rate
15.40	**Tea**	

16.10	Anthony du Vivier and Richard B.Stoughton	An animal model for screening topical and systemic drugs for potential use in the treatment of psoriasis
16.30	R.Marks	Increased DNA and protein synthesis in the involved and uninvolved skin of patients with psoriasis
16.50	P.Holt and R.Marks	Epidermal structure and metabolic activity in acromegaly

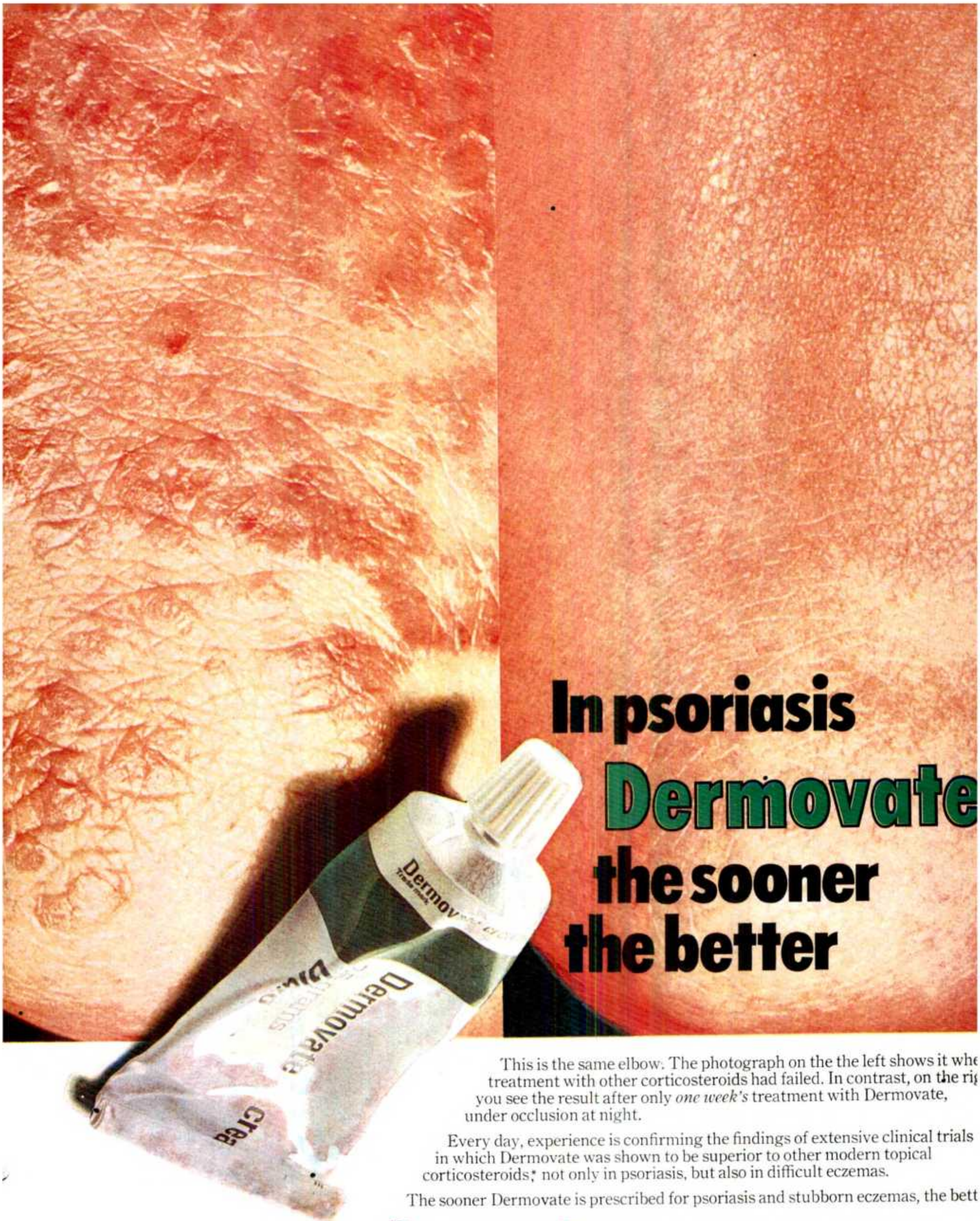
Afternoon Social Programme

14.00	Coaches leave Chemical Laboratory for Ely
16.00	Tea in Kings' School, Ely
17.00	Coaches leave Ely for Cambridge
Golf	The Dowling-McGaw Cup will be held at the Flempton Golf Club
Tours	Guided tours of the colleges
19.30 for 20.00	The annual dinner in Trinity College

Saturday 5 July

Clinical Meeting. New Addenbrooke's Hospital, Hills Road, Outpatient Department and No. 1 Lecture Theatre, Chemical Laboratory, Lensfield Road, Cambridge

09.00	Meet at west car park of the Chemical Laboratory for transport to New Addenbrooke's Hospital
09.30	Patients may be seen in the Outpatient Department of New Addenbrooke's Hospital (Clinics 5, 6 & 7)
10.30	**Coffee** in the Outpatient Hall of New Addenbrooke's Hospital
11.00	Buses will leave New Addenbrooke's Hospital for Chemical Laboratory
11.30	Discussion in No. 1 lecture theatre, Chemical Laboratory, Lensfield Road
13.00	**Lunch** in nurses' dining room, Old Addenbrookes' Hospital, Trumpington Street



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British Journal of Dermatology (1975) **93**, Supplement **11**, 9.

SUMMARIES OF PAPERS

Ochronosis and colloid milium from hydroquinone in man: 50 cases, with pathology and electron microscopy

G.H. FINDLAY

University of Pretoria, South Africa

Various skin blanching agents have been used by black women on the face for decades, but in 1966 the hydroquinone type was widely introduced in higher concentrations in an adhering cream base.

Over the past 5 years, gradually increasing numbers of cases showing facial ochronosis and pigmented colloid milium have been seen, some of whom also developed ochronosis of the ear cartilage. These features were traceable to the forceful inunction, over periods often of years, of hydroquinone face creams along with sun exposure.

The characteristic sequence of events was a loss of the bleaching effect, with an apparently full recovery of epidermal melanocyte function. Then the subpapillary fibroblasts in focal areas were activated to produce an array of new types of fibres—broad, straight collagen bundles that disintegrated into colloid masses, solar elastosis, ochronosis of elastic type fibres, diffuse ochronosis of collagen and colloid, and certain hybrid fibres. In principle the changes were identical to those of alkaptonuria.

The movements of ochronotic pigment have led to some novel views on the origins and behaviour of these extracellular materials and of the cell types concerned with them.

The treatment of hypermelanosis with 4-isopropylcatechol

STANLEY S. BLEEHEN

Hallamshire Hospital and University of Sheffield

This is to report the clinical use of 4-isopropylcatechol (4-IPC) for the treatment of hypermelanosis. This compound was found to be more potent and more consistent in its depigmenting effect than the known 'skin bleaching creams' containing hydroquinone or the monobenzyl or monomethyl ethers of hydroquinone.

Over the past 6 years, sixty-eight patients have been treated with 1% and 3% 4-IPC. Fifty-four of these patients had melasma; the others a variety of disorders of pigmentation. Two-thirds were significantly improved, with bleaching away of much of the excess pigment. The incidence of side effects due to skin irritation was 18% with 3% 4-IPC and 8% with 1% 4-IPC. Four patients developed an allergic contact dermatitis and one confetti-like areas of depigmentation in the 4-IPC treated areas. Light and electron microscopic studies showed that in the 4-IPC treated areas there was a loss of melanocytes but keratinocytes and Langerhans cells were unaffected. Melanosomal complexes often containing many melanosomes were found more frequently in the keratinocytes of 4-IPC treated areas than in keratinocytes of the respective control untreated hyperpigmented areas.

4-Isopropylcatechol is a potent depigmenting agent and is of use for the topical therapy of hypermelanosis. However, like hydroquinone and other substituted phenols it is irritant to the skin.

A controlled study of vitamin E treatment in epidermolysis bullosa

ROWAN H.ADAMS, R.A.MAIN AND R.A.MARSDEN

Aberdeen

There have recently been several reports that vitamin E is of no benefit in the treatment of epidermolysis bullosa. In order to verify this eight patients with dystrophic epidermolysis bullosa (2 recessive, 6 dominant) took part in a double blind crossover trial with vitamin E (d.l.—alpha tocopheryl acetate, Ephynal-Roche). Patients with active blistering of the skin were selected.

After a control period of 2 weeks each patient took one of the paired coded preparations, either placebo or active ingredient chosen at random, for 8 weeks followed by the remaining preparation for a further 8 weeks. The dosage of vitamin E was 1200 mg daily in three divided doses. Patients kept a record of all new blisters which appeared during the trial period. Each patient was also seen every 2 weeks by one of the authors. Blood was taken for serum vitamin E estimations at the end of the 2nd, 6th and 14th weeks.

At the end of the trial the code was broken and revealed that there was no significant difference in effect between the vitamin E and the placebo preparation.

Insofar as we are aware, this is the largest controlled study of the use of vitamin E in epidermolysis bullosa that has been reported to date. It is unfortunate that we were unable to show a beneficial result.

Acne and eczema—a familial study

K.LIDDELL

Southampton University Hospital Group

The incidence of eczema in patients with acne was compared with that in a control group who did not suffer from acne. Age and sex matched controls were selected from 1500 pupils of local schools and colleges, with those free of acne being assessed for evidence of eczema. Eczema was present in 21·7% of the control group but in only 6% of acne patients, a highly significant difference ($P<0{\cdot}005$).

More than 200 families of acne and non-acne subjects were assessed for evidence of acne, eczema and asthma. 18·8% of parents of non-acne subjects had eczema compared with 7% of parents of acne patients ($P<0\cdot001$). 17·2% of siblings of non-acne patients had eczema but 6·8% of siblings of acne patients had eczema, ($P<0\cdot01$). A separate group of eczema patients in the acne-prone age range was also studied; only 8% were found to have acne whereas 50% of pupils examined had acne of varying degree.

In all groups the incidence of asthma and of psoriasis were recorded and there was a marginally significant increase in asthma in the non-acne subjects ($0\cdot05<P<0\cdot1$). However, there was no significant difference in the incidence of psoriasis in any group.

These results indicate an inverse relationship between eczema and acne.

	Incidence of eczema (%)	Statistical significance
Acne patients	5·9	$P<0\cdot005$
Controls (non-acne subjects)	21·7	
Parents of acne patients	7	$P<0\cdot001$
Parents of non-acne controls	18·8	
Siblings of acne patients	6·8	$P<0\cdot01$
Siblings of non-acne controls	17·2	

Rosacea, migraine, Dixarit

W.J.CUNLIFFE, S.G.TAN AND E.TEBBS

The General Infirmary, Leeds

Facial flushing may be troublesome in patients with rosacea; vascular changes including cerebral vasodilation also occur in migraine. Could there be an association between migraine and rosacea? A random sample of sixty-five patients with rosacea showed that 43% had migraine and this contrasts with 26% of matched controls ($P=0\cdot05$). Six of the controls with migraine also had marked blushing; if these are excluded from the group then the association between migraine and rosacea is more evident ($P=0\cdot005$). Dixarit is known to help migraine by reducing the vascular reactivity; we therefore investigated the effect of Dixarit (2 tablets twice daily) in sixteen patients with rosacea; thirteen obtained a reduction in the severity and frequency of facial flushing by at least 50%.

The effect of therapy was apparent within a few days and lasted for 1 week after stopping treatment. Objective assessment of therapy was obtained by measuring facial skin temperature before and during treatment. The resting temperature was lower during treatment than it was before therapy (by 1–2°C): vasodilatation induced by Trinitrin was significantly greater in patients before treatment than during treatment ($P = 0{\cdot}005$).

These data indicate that rosacea and migraine are associated and that the vascular abnormality of rosacea can be improved with Dixarit.

A follow-up of tetracycline treated rosacea (with special reference to rosacea keratitis)

ARTHUR KNIGHT AND C.F.H.VICKERS

Liverpool

There are no published data on the natural history of rosacea. Clinical impressions suggest that the disease lasts for many years with relapses and remissions. Sneddon (1966) first reported the beneficial effect of systemic tetracycline and the present study was initiated to observe the long term results of treatment.

Seventy consecutive patients with rosacea were treated with systemic tetracycline, 250 mg twice daily, for 6 months. The drug was then slowly withdrawn over the next month. Sixty-eight patients cleared with treatment. During withdrawal of the drug seventeen relapsed immediately. The other patients were followed up for a maximum of 4 years at which time the overall relapse rate was 69%. The serum tetracycline levels were not significantly different in the two patients who failed to respond. Six patients had rosacea keratitis and responded with dramatic improvement within 1 month. Symptoms recurred as the drug was withdrawn. It is suggested that rosacea patients with keratitis should receive early and prolonged tetracycline medication.

REFERENCE

SNEDDON, I.B. (1966) A clinical trial of tetracycline in rosacea. *British Journal of Dermatology*, **78**, 649.

Pemphigus induced by D. penicillamine: an 'experimental' pemphigus

J.HEWITT, M.BENVENISTE, AND M.LESSANA-LEIBOWITCH

Clinique Tarnier, Paris 6

Nine cases of pemphigus induced by D. Penicillamine given for rheumatoid arthritis have been observed since the first case (Prof. Degos) treated for Wilson's disease. All the patients were treated in Paris and had received D. penicillamine from three different sources—one French, two German.

The pemphigus was most commonly of the seborrhoeic type, with pemphigus vulgaris in two cases and an atypical pseudo-dermatitis herpetiformis in one.

There was evidence of cytotoxicity as shown by cytological and biological tests. Some were easily cured after stopping the D. penicillamine. Others ran the usual course of the disease.

These cases of iatrogenic pemphigus, the mechanism of which will be discussed, throw new light on the aetiology of pemphigus.

Eosinophilic spongiosis, a clinical, histological and immunofluorescent correlation

A.G.KNIGHT,* T.J.DELANEY† AND M.M.BLACK‡

Department of Histopathology, Institute of Dermatology, WC2, Department of Dermatology,† St George's Hospital, SW1, St Thomas' Hospital,‡ London*

Eosinophilic spongiosis is a rare histological finding first reported by Emmerson & Wilson Jones (1968). They described seven patients with a widespread bullous eruption in whom early biopsies showed eczematous spongiosis and infiltration of the epidermis with eosinophils. Subsequent biopsies and the clinical course revealed them to have pemphigus. Immunofluorescent facilities were not available at that time.

The present study concerns the investigation of five patients, all female, aged between 56 and 73 years. The clinical presentation was of a widespread pruritic eruption with grouped tense bullae. A confident clinical diagnosis of bullous pemphigoid was made. In two cases the initial histology showed sub-epidermal bulla formation consistent with pemphigoid. However, further biopsies revealed small foci of spongiosis containing eosinophils, and serial sections showed mid-epidermal blister formation. Immunofluorescent studies gave identical results in three patients. Direct immunofluorescence showed strong intercellular fluorescence to IgG whilst indirect immunofluorescence was consistently negative. Case 4 had negative direct immunofluorescence but intercellular antibodies were detected in the sera to a titre of 1/160. Case 5 showed no immunopathological evidence of pemphigus despite repeated testing over 6 months.

The immunopathological evidence indicates that most patients with eosinophilic spongiosis do indeed have pemphigus. However the possibility cannot be entirely excluded that Case 5 may be different and analogous to a condition recently delineated in children (benign chronic bullous dermatosis of childhood) (Bean *et al.*, 1971) which closely resembles bullous pemphigoid but in which the immunofluorescent findings are consistently negative.

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Personal experience with clofazimine in the treatment of leprosy

R.D.AZULAY

Universidade Federal Fluminense and Universidade do Estado da Guanabara, Rio de Janeiro, Brazil

We have treated twenty cases of advanced lepromatous leprosy with clofazimine. They were adults, predominantly male, all Brazilian except for one Portuguese, who had been afflicted by leprosy for 1–28 years, most of them long-standing cases (sixteen for over 10 years); fifteen of the twenty were sulphone resistant cases.

They were treated during 5 years; the initial daily dose was 200 mg, which was later decreased to 100 mg.

Clinical. Eighteen cases had a complete regression of the skin and mucosal lesions; in the other two the regression was marked.

Bacterioscopy. All the cases became negative.

Histopathology. The lepromatous infiltration practically disappeared; brownish pigmentation was observed in both the epidermis and dermis.

Leprosy reaction. Before treatment 11 of the 20 cases had a positive reaction, which disappeared with the treatment.

Side effects. They were minor. Reddish and dark pigmentation and ichthyosis-like lesions were observed as well as some infrequent dyspeptic symptoms.

Cutaneous aspects of mongolism—a clinical study

P.W.GRANT AND D.I.MCCALLUM

Raigmore Hospital, Inverness

The study of Trisomy 21 has attracted considerable attention since Lejeune, Gauthier & Turpin (1959) first described the genetic defect responsible for the condition, i.e. three no. 21 chromosomes, instead of the usual two, giving rise to a total of forty-seven chromosomes in place of the normal forty-six.

Several authors have reported on the skin changes in Trisomy 21. These include ichthyosis, lichen simplex, keratosis palmaris, cheilitis and blepharitis. These reports have been quoted in the major textbooks of dermatology including Rook *et al.* (1972).

The authors had the opportunity to examine forty-two such patients, twenty-four males and eighteen females, in all of whom the diagnosis of Trisomy 21 had been confirmed genetically.

Clinically, ichthyosis was apparent in a mild degree in only one male. All twelve males over the age of 15 years had a condition which was clinically seborrhoeic eczema/dermatitis of the pre-sternal and interscapular regions, lower abdomen, lumbar and buttock areas and upper thighs, and on histology showed a non-specific inflammatory reaction and none of the features of ichthyosis. Apart from mild acne in two patients, the females showed no evidence of this eruption.

The authors agree with Du Vivier & Munro (1974) that alopecia areata (seven males) was more common than in the general population.

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Immunity in herpes simplex

R.W.Gange, C.H.P.Bradstreet, A.De Bats, J.R.Park and E.L.Rhodes

St Helier Hospital, Carshalton

Cellular immune responses and circulating antibody levels to herpes simplex virus were examined in patients with recurrent herpes simplex infections and controls.

Mixed leukocyte migration inhibition by herpes simplex antigen was less in affected patients than in controls but serum antibody levels were higher.

There was no significant difference in leukocyte migration between patients with active or recent lesions and other herpes subjects and the response in the mixed leukocyte migration test to phytohaemagglutinin was similar in patients and controls.

The data presented suggest that a localized defect in cell mediated immunity to herpes simplex virus may exist and be responsible for recurrent herpes simplex infections.

Are moles just naevus cells?

C.B.Bentley-Phillips and R.Marks

The Welsh National School of Medicine, Cardiff

Traditionally moles are thought of as the result of faulty development of pigment forming cells derived from the neural crest. We have been impressed by the well recognized but poorly documented epidermal hyperplasia that accompanies many moles. In this study we have attempted to characterize these epidermal changes in order to learn more concerning dermo–epidermal interactions and the histogenesis of moles.

Two hundred moles have been examined histologically and the degree and type of epidermal hyperplasia recorded and correlated with the site, arrangement and density of the naevus cells.

In addition, thirty of the moles were examined autoradiographically after *in vitro* incubation for 2½ h in the presence of tritiated thymidine. Resemblances to the epidermal architecture of seborrhoeic warts were noted in 60% of the moles examined. This kind of alteration was seen when there were aggregates of naevus cells at the dermo–epidermal junction and in the upper dermis. Epidermal thinning was evident in 10% of the moles and was seen when there were dense aggregates of naevus cells throughout the dermis. Naevus cells as well as epidermal cells became autoradiographically labelled by the tritiated thymidine but there was poor correlation between epidermopoiesis and 'naevopoiesis' when assessed by this technique.

It is suggested that the characteristic epidermal change is 'seborrhoeic wart like' and generated from a stimulus in the upper dermis.

Naevus spilus: a light and electron microscopical study

R.A.J.EADY AND T.C.COWEN

Institute of Dermatology, Homerton Grove, London

The relationship between the pigmented macules of neurofibromatosis and naevus spilus is not fully understood, although giant melanin granules have been found in both conditions.

In this study, naevi spili and café-au-lait macules, occurring together in two patients, were examined by light and by electron microscopy. Apart from the abnormal pigmentation, neither patient showed signs of neurofibromatosis.

In both types of lesion, there was an increase in epidermal melanin content, and in the number of basal clear cells. Additionally, in naevus spilus, small nests of naevus cells were present in the junctional zone and in the superficial dermis. Giant melanin granules, usually spherical but occasionally ellipsoidal in shape, and up to 4 μm in diameter, were found in melanocytes, keratinocytes, naevus cells and macrophages. The giant granules in naevi spili and in café-au-lait macules appeared to be identical, and were composed of concentric shells of vesicular structures which became melanized from the centre outwards. Normal size (0·25 × 0·5 μm) ellipsoidal melanosomes appeared alongside the giant granules and were dispersed within the keratinocytes in large membrane-bound complexes. Small unmyelinated nerve terminals were frequently seen in the papillary dermis, usually in the vicinity of melanocytes or naevus cells.

These findings have shown that naevi spili and café-au-lait macules are morphologically similar, and that the 'spottiness' in the former may simply result from naevus cell activity. Furthermore, giant melanin granules in café-au-lait macules are not necessarily a marker of neurofibromatosis.

Identification of lymphoid cells in the cutaneous infiltrate of skin reticuloses and lichen planus

R.S.-H.TAN, J.P.HAYES AND N.A.BYROM

Westminster Hospital, London

Sixteen patients were investigated using a new technique by which viable cells can be freed from skin biopsies after treatment with collagenase. In five patients with mycosis fungoides and two with the Sézary syndrome the most lymphoid cells, with morphological characteristics of Sézary cells, were liberated from the skin of both Sézary patients. However in two of four patients with malignant lymphoma, B cells predominated. Our findings support the concept that mycosis fungoides and the Sézary syndrome are disorders of T cells, and that some non-Hodgkin lymphomas are disorders of B cells.

In the skin lesions of five patients with lichen planus, T cells predominated; very few B cells were detected. If similar T cell predominance is found in other dermatoses, the skin would seem to be a non-lymphoid site for the 'homing' of T cells, and could even be considered as an organ of immunological surveillance.

International mortality from bullous diseases since 1950

J.A.SAVIN

Royal Infirmary, Edinburgh

National mortality figures hold information about our overall success in treating bullous disorders which cannot be found elsewhere. The official data since 1950, for England and Wales, the United States, France, Japan, Scotland, Ireland and Denmark, have been analysed after corrections for changes in the population structure and in the International Classification of Disease.

A steep fall in deaths occurred in England and Wales between 1952 (121 deaths) and 1955 (55 deaths). The low rate persisted from 1955 to 1962 (40 deaths) when it fell to a steady even lower level lasting until the present. The United States figures showed a sharp drop in mortality starting about 2 years earlier (from 283 deaths in 1950 to 150 deaths in 1954), followed by a gradual fall till the present. Mortality in Scotland, Ireland, and Denmark has followed the same general pattern but the figures from France, after a steady fall between 1951 (68 deaths) and 1965 (29 deaths), have risen again until 1971 (62 deaths). Deaths have not fallen in Japan but the data are difficult to interpret.

The timing of the improvement in mortality in England and Wales, and in the United States, fits well with the introduction of systemic steroids into general use. A fall to about one third of the pre-steroid level is in general agreement with the improvement recorded in smaller personal series from several centres.

Immunopathological findings in herpes gestationis

J.A.CARRUTHERS* AND M.M.BLACK†

**Department of Histopathology, Institute of Dermatology, London WC2, and †Department of Dermatology, St Thomas' Hospital, SE1.*

Herpes gestationis is a rare bullous dermatosis of pregnancy and the puerperium. Most authorities believe that it is a distinct entity, although it may closely simulate erythema multiforme or dermatitis herpetiformis. The advent of immunopathological techniques has enabled the latter two conditions to be distinguished, but because of the rarity of herpes gestationis few cases have been studied using immunofluorescent techniques.

Using direct immunofluorescence, Provost & Tomasi (1973) demonstrated deposition of complement on the basement membrane (BM) in two cases of herpes gestationis, and Buskell, Jordon & Goltz (1974) reported the presence of a circulating IgG antibody to basement membrane in a further case.

We have recently investigated three patients with severe herpes gestationis (aged 20–31 years). In all three patients the eruption persisted into the puerperium (in one for as long as 13 months post-partum). In all three cases direct immunofluorescence of peri-bullous skin revealed a linear BM zone band with C3 component of complement. In two cases an IgG band was also demonstrated on direct immunofluorescence but in none could a circulating antibody of IgG type be demonstrated against the basement membrane. Two of the three cases have been shown to have a circulating factor capable of depositing complement on the basement membrane in normal skin.

These results indicate that herpes gestationis is not related to erythema multiforme or to dermatitis herpetiformis. Herpes gestationis appears to be allied to bullous pemphigoid in that these diseases have in common activation of the complement pathways at the level of the basement membrane.

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Lichen planus: investigations of carbohydrate metabolism and the HLA system

N.J.LOWE AND A.G.CUDWORTH, INTRODUCED BY C.F.H.VICKERS

Departments of Dermatology and Medicine, University of Liverpool

Previous studies in patients with lichen planus have shown impairment of carbohydrate metabolism (Jolly, 1972; Powell *et al.*, 1974).

We have further investigated patients with this condition in order to determine: (1) The incidence of the abnormal carbohydrate metabolism. (2) The incidence of any impaired insulin response to a glucose load. (3) Any association with HLA phenotypes.

METHOD

Patients were randomly selected but subjects with a positive family history of diabetes mellitus or taking systemic corticosteroids were excluded from glucose tolerance testing. HLA phenotypes were obtained on all patients.

RESULTS

(1) Thirty-six patients agreed to have a 100 g oral glucose tolerance test. Of these, eleven (31%) had unequivocal evidence of chemical diabetes; a further five subjects had a single abnormal elevated peak or high 2 hour blood sugar (B.D.A. classification).

Symptomatic maturity-onset diabetes mellitus was present in four patients.

(2) The serum insulin response to glucose in the patients with chemical diabetes was consistent with that found in maturity-onset diabetes mellitus.

(3) Forty-nine patients were HLA typed. HL-A 5 was present in ten of forty-nine patients (20·4%) compared with twenty-seven of 300 (9·0%) control subjects.

On further analysis HL-A 5 was present in six of fifteen (40%) of the group in which chemical or symptomatic diabetes and lichen planus co-existed.

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Challenge test battery in chronic urticaria

ROBERT P. WARIN AND RUTH J. SMITH

Bristol Royal Infirmary

A number of substances, many of which may be present in foods, have an exacerbating effect in patients with chronic urticaria. This has been shown by a number of workers, notably Juhlin & Michaëlsson (1972, 1973). In an individual patient with chronic urticaria, the only way to determine whether or not any of the substances have such an exacerbating effect is to administer test doses. An attempt has been made to develop a battery of the likely substances, which can be administered in a routine way as part of the investigation of a patient with chronic urticaria. Various chemicals, doses and timings have been tested, and the first battery elaborated is as follows:

A Control candida (liquid)
B Candida (liquid)
1 Control
2 Tartrazine 1 mg with lactose
3 Tartrazine 10 mg with lactose
4 Control
5 Sodium benzoate 50 mg
6 Sodium benzoate 500 mg
7 4-Hydroxybenzoic acid 50 mg
8 4-Hydroxybenzoic acid 200 mg
9 Control
10 Yeast extract 0·4 g
11 Tartrazine 10 mg with kaolin
12 Tyramine 100 mg
13 Penicillin 1 mg
14 Penicillin 125 mg
15 Aspirin 150 mg
16 Aspirin 600 mg

Tests 3, 6, 8, 14 and 16 should not be taken if the weals increase with the previous dose.

The substances and the control lactose were incorporated into identical capsules, labelled, and were administered every morning consecutively. The patients had a chart, and made a note of the severity of the weals on every day. If there were any positive results, the substances responsible, together with suitable controls were readministered in an altered order. If there was again an exacerbation, the particular substance was regarded as giving a positive result. The patients all had typical chronic urticaria present for at least 2 months, and other patterns of urticaria including all the physical urticarias were excluded. Antihistamine therapy was continued if, from the aspect of clinical management, this was necessary, but the dose was adjusted as far as possible so that the patient had some relief, but weals were still developing. This same dose was then continued throughout the whole test period. Five patients were tested after admission to hospital but the remainder were Out Patients. After investigating the first forty patients, the battery was changed to the following:

1 Control
2a Tartrazine 1 mg with lactose (instead of 2 if history of asthma)
2 Tartrazine 10 mg with lactose
3 Control
4a Sodium benzoate 50 mg (instead of 4 in children under 10)
4 Sodium benzoate 500 mg
5 Control
6a 4-Hydroxybenzoic acid 50 mg (instead of 6 in children under 10)
6 4-Hydroxybenzoic acid 200 mg
7 Control
8 Yeast extract 0·6 g
9 Control
10 Penicillin 0·5 mg (not to be given if any history of severe penicillin reactions)
11 Control
12 Aspirin 100 mg
13 Aspirin 600 mg (not to be given if history of asthma or severe aspirin reactions)

The reasons for the modifications will be explained. It is likely that the battery will need to be altered in the future. For example 4-hydroxybenzoic acid might be left out, and other substances may well have to be added. The results in the first 111 patients are summarized as follows:

Total no. of patients	No exacerbations	Aspirin	Sodium benzoate	4-Hydroxy benzoic acid	Tartrazine	Yeasts and candida	Penicillin
111	45	45	12	5	14	15	17

It will be noticed from the numbers of reactions recorded that some patients showed an exacerbation with two or more substances. This occurred particularly with aspirin and penicillin (six patients) and aspirin and tartrazine (five patients).

Diet sheets have been prepared, which reduce as far as possible the content of salicylates, benzoic acid preparations, tartrazine, and yeasts. The patients who gave a positive reaction to one or more substances were given the appropriate diet and the results assessed after 2 months. If improvement had occurred, the diet was continued, and the patients instructed gradually to relax the diet regime over the course of the next few months. Of the forty-seven patients in whom the diet investigations have been completed, thirty-five either cleared or improved considerably, and in three there was no effect. Five were impossible to assess and four failed to keep to the diets satisfactorily. It must, however, be pointed out that many patients with chronic urticaria improve spontaneously after a period of some 3–9 months.

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Trichotillomania: a pathogenic interpretation of histologic changes in the pilosebaceous unit

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Seven cases of trichotillomania have been investigated histologically. Three hundred serial sections were obtained from each specimen.

Histopathologically lesions can be divided into two categories, depending on more or less complete plucking of the hairs.

(1) When *plucking* is *complete* (empty hair canals), clumps of sebaceous glands are sometimes torn off, and their cells can be found between cells of the horny layer of the epidermis. This is followed by an isomorphic proliferative reaction at the different levels of the pilosebaceous unit. Follicular plugging results from a tortuous and extensive keratinization of ostial and infundibular cells of the external root sheath (with melanin casts). A 'basaloid' proliferation from the sheaths in the lower part of the follicle evolves as in a normal catagen phase.

(2) *Incomplete pulling* results in the development of severe clefts of the hair bulb, leading to the consequent formation of dystrophic and soft hairs (Miescher's trichomalacia) and to the release of melanin in the papilla.

Some histological features (some of which were not described in previous papers (Mehregan, 1970; Muller & Winklemann, 1972)) are *specific* for trichotillomania (i.e. empty hair shaft, sebaceous gland extrusion, bulbar clefts, trichomalacia) whereas others are *non-specific* (i.e. release of melanin pigment, proliferation of undifferentiated cells from the sheaths), since they may be encountered in alopecia areata or in some pilary agenesies.

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A study of persistent light reaction in quindoxin and quinine photosensitivity

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Quindoxin (quinoxaline 1,4-di-N-oxide) when added to animal feedstuffs acts as a growth promoting factor. It has also been shown to be responsible for photocontact dermatitis in human subjects (Scott & Dawson, 1974). Quinine will also cause 'photosensitivity' and contact allergic dermatitis. We have studied two male pig farmers with quindoxin photosensitivity, and one industrial chemist with quinine photosensitivity, from the point of view of persistent light reaction and the apparent similarity to the chronic photosensitivity dermatitis and actinic reticuloid syndrome (Ive *et al.*, 1969).

Materials and methods. (a) In vivo *studies*. The action spectrum in each subject was defined by monochromator irradiation (Mackenzie & Frain-Bell, 1973) and photopatch testing carried out to concentrations of quindoxin (0·1%, 0·05%, and 0·01%) in PMF followed 24 h later by irradiation with filtered blacklight long UVR (300–355–400 nm). Similar studies were done in a normal subject and in a farmer with a photodermatosis who had not been exposed to quindoxin; dosage levels of irradiation and time intervals prior to irradiation were varied. The quinine photosensitivity subject was similarly investigated.

(b) In vitro *studies*. Washed human red blood cells and mouse peritoneal macrophages in short term culture (Johnson, 1974) were used to study the phototoxic potential of quindoxin. Phototoxicity in these model systems leads to haemolysis and cell death respectively.

'Blacklight' fluorescent tubes were used for irradiation providing a broad band of long wavelength ultraviolet radiation, wavelengths 305–395 nm, peak 355 nm. Variations in dose of radiation and concentration of quindoxin from 0·01 to 1·0 mg per 100 ml were studied.

Results. Persistent light reaction involving the ultraviolet and visible wavelengths was demonstrated to a variable extent in all three subjects along with positive photopatch tests to quinine and quindoxin which with the latter occurred with a concentration as low as 0·01% and with a dosage level of long UVR down to 42 mJ/cm^2. The negative photopatch test response in the control subjects and the results of the *in vitro* studies tend to exclude a phototoxic and to favour a 'photo-allergic' reaction.

Discussion. On clinical and photobiological grounds quindoxin and quinine photosensitivity appear to produce in human subjects an effect in the skin similar to that seen in the state of persistent light reaction of the chronic photosensitivity dermatitis and actinic reticuloid syndrome (Frain-Bell *et al.*, 1974).

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A preliminary report of a vascular abnormality occurring in men engaged in the manufacture of polyvinyl chloride

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Angiosarcoma of the liver and acro-osteolysis (Raynaud's phenomenon, lytic lesions of bone and sclerodermatous skin change) are now accepted as occupational hazards in men engaged in the process of polymerisation of vinyl chloride to polyvinyl chloride (PVC). In 1974, Lange *et al.* described a multisystem disease with fibrosis of the lungs, liver and spleen in addition to acro-osteolysis.

In the last year, thirty men from a local PVC plant have presented with symptomatic complaints of coldness and paraesthesiae of their hands and/or feet. Additional symptoms include undue fatigue, muscle and joint pains, dyspnoea and impotence. A severe but variable acrocyanosis of the hands and feet has been observed in at least ten men. Only two cases show radiological bone changes and these are minimal. Sclerodermatous skin change is not present. Skin histology reveals fragmentation of the elastic tissue in the dermis and abnormal staining of the collagen.

Arteriograms in the more severely affected men demonstrate narrowing of digital vessels. Finger blood flow and skin temperature fall dramatically when the patient is cooled.

Immunological investigations in twenty cases have shown cryoglobulins and/or cryofibrinogen in fourteen. Respiratory investigations suggest a perfusion impairment which is as yet unexplained.

The ten most severely affected men are quite disabled by the changes in their hands.

The purpose of this preliminary report is to present the clinical manifestations of a vascular abnormality which may arise in men employed in the manufacture of PVC.

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The periodontal membrane in systemic sclerosis

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Radiographic examination of the jaws and teeth was carried out on thirty patients suffering from systemic sclerosis and thirty control subjects matched by age and sex.

In systemic sclerosis 21% of 590 teeth at risk had widening of the periodontal membrane compared with 4·9% of 759 teeth in controls. Of the affected teeth the whole root was involved in 70% and the coronal third of the root in 30% in systemic sclerosis compared with total root involvement in 40% and coronal third involvement in 60% in controls. Thickening of the periodontal membrane occurred in 10·3% of teeth at risk in a series of sixteen patients with chronic periodontitis but clinical evidence of this disorder was apparent in the patients.

In systemic sclerosis not all teeth are affected in any subject but there is no evidence that molars and premolars are more involved than incisor teeth as has been suggested. There is no increase in mobility of the teeth.

Thickening of the periodontal membrane in systemic sclerosis is unrelated to the duration of Raynaud's phenomenon, calcinosis, involvement of the heart, chest, gastrointestinal tract, or kidney, or to the presence or absence of antinuclear factor. When marked it tends to be associated with other system involvement but can occur when only the skin is involved. There is no association between thickening of the periodontal membrane and a poor prognosis or fatal outcome.

Widening of the periodontal membrane was not demonstrated in two patients with generalized morphoea or in three patients with localized morphoea, two of whom had hemiatrophy of the face.

Case report

Fingernail growth in peripheral pustulosis

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The clinical entity of peripheral pustulosis has similar clinical and histological findings to the acral pustulation of hands and feet that sometimes occurs in the course of psoriasis vulgaris. In consequence when such pustulation occurs in the absence of psoriasis on other parts of the body it is often called pustular psoriasis; this diagnosis remains unproven.

In view of the fact that normal nails of subjects with psoriasis vulgaris grow significantly faster than those of non-psoriatics (Dawber, 1970), we have measured fingernail growth in a group of patients with peripheral pustulosis and compared the results with normal and psoriatic subjects. The results show no significant difference between the rate of nail growth in normal and peripheral pustulosis subjects, both these groups growing significantly slower than psoriatic nails.

These results together with recent HL-A antigen studies (Svejgaard *et al.*, 1974) suggest that peripheral pustulosis is aetiologically different from psoriasis vulgaris.

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Reactivation of Herpes virus hominis (HVH) infection in mice treated with cyclophosphamide and prednisolone sodium phosphate (Codelsol)

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Model. A highly reproducible mouse (Webster Schneider mice) model system, in which linear zosteriform skin lesions are produced following intradermal inoculation of HVH, has been used throughout these studies (Robinson & Dover, 1972).

Reactivation. Groups of previously infected mice that had recovered and remained well have been challenged at least 8 weeks after primary infection with cyclophosphamide (CPA) and Codelsol using the following schedule: Day 1 200 mg/kg CPA, Day 2 200 mg/kg Codelsol, Day 3 200 mg/kg Codelsol plus epilation of originally infected site, Day 4 200 mg/kg Codelsol, Day 5 200 mg/kg CPA.

The incidence of reactivation is about 60%, which is much higher than that obtained in the only other comparable study (Underwood & Weed, 1974).

Conclusions. Useful animal models for the study of the pathogenesis of HVH, with and without the use of immunosuppressants, have been established. A model with a high incidence of reactivation is described. This model is being used to study the reactivation process in these animals using histological and immunofluorescent techniques (and perhaps electron microscopy later). Any available results will be discussed. These studies serve to underline the fact that the course of viral infections may be dramatically modified by the action of immunosuppressant agents.

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Fluctuations in the blood flow of forearm skin

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The oxygen tension at the skin surface, when the atmospheric oxygen supply is excluded, may be measured polarographically. If the experimental subject is seated in a room at 25°C and his arm is in a box at 33°C, spontaneous fluctuations in skin surface oxygen tension can be seen because of fluctuating blood flow in the superficial skin vessels (Evans & Naylor, 1967). An array of cathodes in a single electrode has been used to measure the fluctuations in blood flow which are occurring simultaneously at seven different points in the skin. If a long recording of such fluctuations is made, spectral analysis of the fluctuations shows that most of the power is concentrated at the lower frequencies but reveals no clearly defined preferred frequency. It is clear however from the fluctuation patterns that recurring bursts of activity do occur with quite well defined periodicities in the range 30 s–5 min. There is a stronger correlation between the fluctuations occurring at points on the skin less than 1 mm apart than between points further apart, although there are clearly some components which are common to points separated by up to 5 mm. These results suggest that the fluctuations may be a result of a common central effect which can then be modified or overridden by peripheral mechanisms controlling blood flow.

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The bacteriology of skin cysts

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The three common types of skin cyst (epidermoid cyst, pilar cyst and steatocystoma multiplex) are easily distinguishable both clinically and histologically. Only epidermoid cysts frequently become inflamed clinically and this is the only type that has a punctum visible on the skin surface. It was decided to investigate the contents of all three types of cyst to discover whether microorganisms were consistently present in any type and if so whether these were the same as those on the body surface.

Cysts were removed intact under local anaesthesia. They were placed in a sterile dry container. They were then broken up using a teflon homogenizer in sterile nutrient broth containing 1% Tween 80. The volume was made up to 10 ml and serial ten-fold dilutions made to 10^{-3} or 10^{-4}. Five drops, each approximately 1/40 ml, of each dilution were placed on blood agar and Sabouraud agar for aerobic incubation and on Brewer's thioglycollate medium (without methylene blue but with 0·1% Tween 80) for anaerobic incubation in a MacIntosh and Fildes jar. Aerobic plates were incubated for 3 days and anaerobic plates for 5–7 days at 37°C. The colonies produced were counted and identified at least to the generic level.

Organisms were only recovered from epidermoid cysts. These were coagulase negative staphylococci and diphtheroids. Corynebacterium acnes and yeasts were not found.

These results support the hypothesis that cysts can only become infected if they are connected with the skin surface. It is surprising that Corynebacterium acnes and the pityrosporum species were not found.

Lipase, hyaluronidase and protease production from bacteria isolated from acne patients

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There is evidence that several exo-enzymes (lipase, hyaluronidase and protease) are important in the pathogenesis of acne and so we have investigated these exo-enzymes in bacteria isolated from acne patients.

C. acnes and *S. epidermidis* were isolated from the skin surface and pilo-sebaceous ducts of four types of skin—normal skin of acne patients and skin bearing blackheads, papules and pustules.

Triolein was used as the substrate for the detection of lipolytic activity, casein for protease activity and ox synovial fluid for hyaluronidase activity.

C. acnes and *S. epidermidis* both produce abundant lipases and proteases but only *C. acnes* produces hyaluronidase. In areas where there is no inflammation, i.e. normal skin and blackheads, the production of these three enzymes appears to be predominantly by organisms residing on the surface of the skin. However, where inflammation is present, such as papules and pustules, these enzymes are found in the pilo-sebaceous duct.

The data indicate that an overall increase in exo-enzyme production is related to inflammatory lesions and in view of the known role of *C. acnes* in the pathogenesis of acne hyaluronidase may be especially important in mediating inflammation.

Keratin hydration and sebum excretion rate

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Hydration of keratin produces a decrease in pilo-sebaceous pore size and so we investigated the relationship between pore size and sebum excretion rate (SER) in an attempt to explain the flare of acne under hydrating conditions. SER measurements (Strauss & Pochi, 1961) were made on symmetrical areas of the upper back. Pore size was measured by surface microscopy. One side of the back, the test area, was then occluded with polythene and the measurements repeated after 22 h occlusion. There was no significant difference between the SER in the control areas; but on day 2 after the polythene was removed the SER on the test side rose significantly ($P = 0{\cdot}01$) to reach a peak between 60 and 90 minutes, and returned to normal levels after 2 h. The rise of SER correlated with the return of the pores to their original size, which took 90–120 min. The results suggest that at the time of keratin hydration, when the duct size is decreased, sebum is prevented from flowing out of the duct at its normal rate. This would cause some build-up of sebum within the pilo-sebaceous unit and thus a rise in intraductal pressure which might accelerate formation of an acne lesion.

An animal model for screening topical and systemic drugs for potential use in the treatment of psoriasis using a new, rapid and simple technique of extracting DNA from epidermis involving hydroxyapatite column chromatography

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A new hairless mouse model system for testing topical and systemic agents for possible therapeutic value in psoriasis has been designed. Ultraviolet light is used to stimulate increased mitosis, increased DNA synthesis and epidermal hyperplasia thus simulating psoriasis. Two standard methods of determining drug antimitotic activity have been used, the mitotic index and autoradiography to compare with the results from a new procedure for assessing DNA synthesis. The latter involves hydroxyapatite, a compound which has a unique property of high affinity for DNA but not for RNA and proteins. Thus DNA may be extracted from the epidermis by loading crude epidermal cell lysate onto a hydroxyapatite column, washing the RNA and proteins through with a low salt buffer whilst the DNA remains bound until eluted with a high salt buffer. The absorbance of the DNA at 260 nm is measured in a spectrophotometer from which may be calculated the amount of DNA present and then the radioactivity of the sample is determined in a liquid scintillation counter. Results show that 2% nitrogen mustard, fluocinonide cream and 1% chlorambucil topically and cyclophosphamide systemically significantly reduce the mitotic index. The effect of the topical agents is shown to be local and not systemic. DNA synthesis for these drugs was similarly decreased using the new method and autoradiography was used to confirm the decreased DNA synthesis for the latter two drugs. The DNA extraction procedure is very quick, accurate and simple and results may be obtained from 24 samples of skin within 5 h. The technique is being used to screen a large number of compounds for topical and systemic antiproliferative properties in the epidermis which may be developed later for use in the human situation

Increased DNA and protein synthesis in the involved and uninvolved skin of patients with psoriasis

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The increased proliferative activity and heightened metabolism of psoriatic epidermis are both well known but unexplained phenomena. It has been suggested by some workers that the epidermis of uninvolved skin of psoriatic patients is similarly but less dramatically affected. For example, Born & Bickhardt (1969) has found that in uninvolved epidermis there is a relatively slow rate of DNA synthesis for individual cells but that overall the amount of DNA synthesized per unit surface area is increased. In this investigation the rate of incorporation of tritiated precursor compounds has been studied in the involved and in the clinically uninvolved skin of patients with psoriasis. The method used is essentially that of Marks, Fukui & Halprin (1971) in which thin sheets of skin (0·4 mm thick) are removed with a keratotome and incubated for short periods in the presence of the precursor under consideration. The radioactivity in the skin fragment is taken as an index of synthetic activity within the epidermis during the period of incubation. It is expressed in units that take into account both the surface area of the skin and the degree of hypertrophy of the epidermis. Using this *in vitro* method it has been possible to determine the rate of incorporation of tritiated thymidine, proline and histidine in the epidermis of twenty normal controls, in the lesions from twelve patients with psoriasis and in the interlesional skin in sixteen patients. Results show that: (i) the rate of DNA synthesis is increased in interlesional skin of patients with psoriasis, (ii) the rate of synthesis varies greatly in individual subjects, and (iii) the rate of DNA synthesis in psoriatic lesions was only twice normal when expressed in relation to unit length of basal layer and surface area (see Table).

	Activity incorporated per h		
	Thymidine units	Proline units	Histidine units
Controls	12·0 ± 1·8	7·9 ± 1·9	5·6 ± 1·2
Psoriatic lesions	21·1 ± 3·5	11·1 ± 2·4	9·5 ± 2·7
Uninvolved areas	18·5 ± 2·2	8·8 ± 1·3	3·0 ± 0·4

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Epidermal structure and metabolic activity in acromegaly

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Many soft tissue structures are involved in the hypertrophy of acromegaly. The dermis becomes thickened (Black, Shuster & Bottoms, 1972) and there is seborrhoea (Burton *et al.*, 1972). In this study we have examined epidermal structure and growth in six patients with acromegaly in order to determine whether this tissue is also involved. Epidermal architecture was studied by measuring mean epidermal thickness (MET), mean epidermal cell size (MECS) and the ratio between the length of the basal layer and of the granular cell layer (B/G). Replicative activity was studied by determining the autoradiographic labelling indices after injection of tritiated thymidine. The rates of incorporation of tritiated precursor compounds by skin slices obtained with a keratotome were also studied. The results show a significant heightening of replicative activity and a significant change in epidermal architecture when compared to a control group of patients.

The results are given in the table below.

	Controls	Acromegalics	Significance
Labelling indices (%)	5·0	8·7	
MET (nos. of cells)	4·52 ± 0·19	3·46 ± 0·11	$P<0{\cdot}001$
B/G ratio	1·12 ± 0·02	1·08 ± 0·03	$0{\cdot}3>P>0{\cdot}2$
MECS (μm dia.)	11·4 ± 0·38	15·28 ± 0·17	$P<0{\cdot}001$

The incorporation of tritiated thymidine, proline and histidine by skin from acromegalic patients showed an increase of between 80 and 100%.

These results did not correlate well with the serum levels of growth hormone, but did with the dermal thickness when assessed radiologically.

It is concluded that the epidermis is profoundly altered in acromegaly, but the degree of change observed cannot be accounted for by the level of serum growth hormone alone.

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CASE REPORTS

(1) Granuloma annulare in Mauriac's syndrome

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J.W., male, aged 8 years. Hospital case no. 27 41 33.

History. He has had diabetes since he was 2 years and 10 months old. Whereas the diabetes has been relatively straightforward to manage, stunting of growth and fatty infiltration of the liver, histologically proven, have occurred; both are normally associated with poorly controlled diabetes.

In January 1975 lesions of granuloma annulare appeared on the left forearm, palm and side of neck and right flank.

Family history. There is no known family history of diabetes or granuloma annulare.

Examination. Typical lesions of granuloma annulare are present in the above-mentioned distribution.

Discussion. The association of juvenile diabetes, stunted growth and hepatomegaly has been referred to as Mauriac's syndrome (Mauriac, 1934). Necrobiosis lipoidica occurs in about 2% of juvenile diabetes, as compared with 0·25% of adults (White, 1959). Granuloma annulare, however, appears to be uncommon in juvenile diabetes in general, but it has been reported in Mauriac's syndrome (Greenwood *et al.*, 1974).

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(2) Pyoderma gangrenosum

DR DAVID GOLDIN AND DR ARTHUR ROOK

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N.B., male, aged 49 years. Grocer. Hospital case no. 11 25 86.

History. He has had outbreaks of septic lesions on the skin since 1948. These healed without scarring. In August 1972 an area of ulceration developed on his right leg. This failed to respond to antibiotics and, over the next few weeks, other lesions appeared on his trunk and limbs. He was admitted to hospital and was extensively investigated. The diagnosis of pyoderma gangrenosum was made. No other significant abnormality was found.

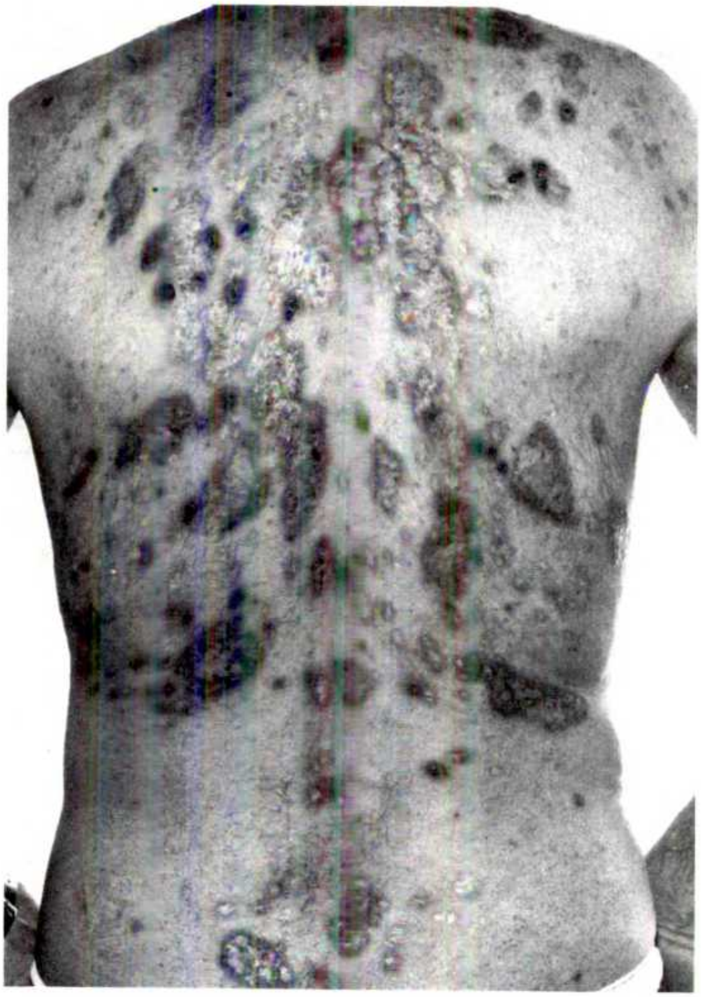

FIGURE 1. Pyoderma gangrenosum lesions on back.

Initial treatment with dapsone was ineffective. He was then given a course of azathioprine, which was also unsuccessful. The lesions began to heal when prednisone was given in a dose of 60 mg a day. He has continued on prednisone since then and the disease was for a time suppressed on a daily dose of 20 mg. Recently he has been getting new lesions and the dose of prednisone has had to be increased. In turn he was put back on azathioprine and then dapsone in the hope of reducing the quantity of steroid needed, but neither of these was beneficial.

Over the course of the illness there have been two intercurrent episodes of quite severe facial acne. The first occurred when he was on prednisone 60 mg a day and the second began when he was given a course of 'blood clearing' tablets by a herbalist. These were found to contain large quantities of a bromide.

Examination. There are ulcers of pyoderma gangrenosum on the trunk and limbs. These are of varying size and age. Large areas of scarring are present.

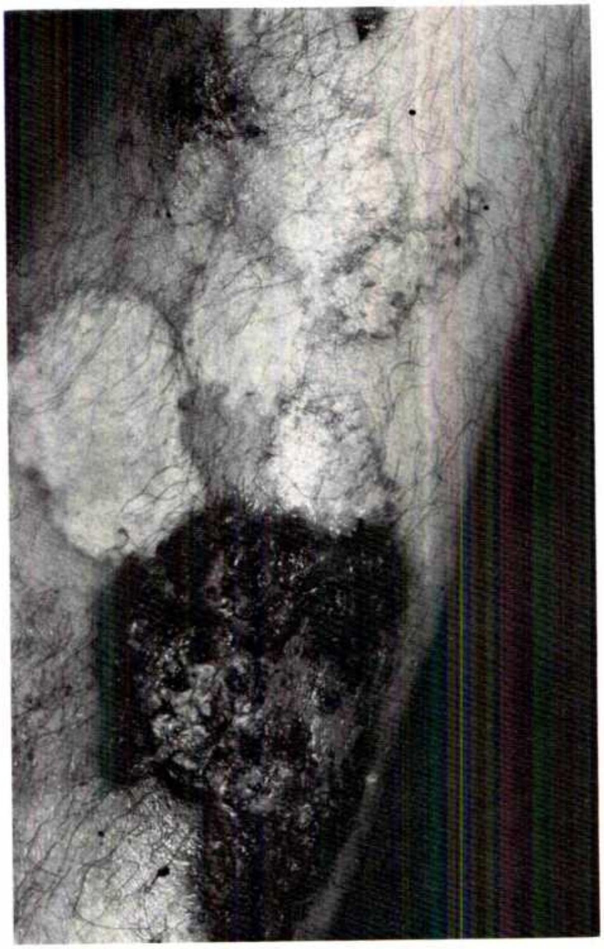

FIGURE 2. Pyoderma gangrenosum lesions on leg.

Investigations. Histology. There is a large ulcer lined with polymorphonuclear neutrophilic leukocytes, granulation tissue and numerous plasma cells. The ulcer extends to the deep dermis. There is widespread surrounding chronic inflammation, oedema and vasodilatation. No fungi are seen.

Routine haematological and biochemical investigations were normal except for a raised eosinophil count of 920/mm^3 early in the illness and an elevated ESR (Wintrobe) of 47 mm/h. Early lesions were usually bacteriologically sterile. Faecal streptococci, coliform bacilli and pseudomonas were frequently isolated from sites of longstanding ulceration. Dinitrochlorobenzene sensitisation was positive.

Other investigations, which were normal, include the following: serum proteins and electrophoresis, serum immunoglobulins, Latex and Rose Waaler tests, L.E. cells, leukocyte alkaline phosphatase score, Australia Antigen, Widal reaction, sigmoidoscopy, chest X-ray, barium studies of small and large bowel.

Discussion. Pyoderma gangrenosum, defined by Perry & Brunsting (1957) as a chronic undermining ulcer of unknown aetiology, may well comprise more than one clinicopathological entity, even when those cases presenting histologically as a necrotizing vasculitis are excluded. Some 40% of cases are associated with ulcerative colitis and occasional cases with hypoglobulinaemia (Marcussen, 1955) or paraproteinaemia (Röckl *et al.*, 1964, Jablonska *et al.*, 1967). Joint symptoms are present in 30%, irrespective of the presence or absence of colitis. Lazarus *et al.* (1972) found abnormal delayed sensitivity in three of four cases.

Our patient has shown no associated disease and he was readily sensitized to dinitrochlorobenzene. The clinical features are those formerly differentiated as phagedena geometrica, but until the pathogenesis of these cases is better understood there would seem to be no adequate grounds for dogmatism in subdividing the 'pyoderma gangrenosum' ragbag, or for not doing so.

It is of interest that Perry & Brunsting (1957) noted that halogen sensitivity may be present.

August & Wells (1974) have recently discussed the treatment of this condition, which is often unsatisfactory. Those cases not associated with discoverable disease run a very chronic course. A patient under our care with similar lesions continued to develop new ulcers over a period of over 30 years, without any evident deterioration in his health (Whittle, 1945).

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(3) Lichen myxoedematosus, Crohn's disease and mucinous ovarian carcinoma

DR RAJ KUBBA, DR N.I.MCNEIL AND DR ARTHUR ROOK

Addenbrooke's Hospital, Cambridge

Mrs M.H., aged 61 years, housewife. Hospital case no. 11 39 50.

Past history. In 1942 she had a thyroidectomy for thyrotoxicosis and in 1961 a right radical mastectomy for carcinoma of the breast.

Present illness. She has suffered from intermittent diarrhoea and abdominal pain since 1955 and a diagnosis of Crohn's disease was finally established in 1972. In September 1974 she noticed a few skin coloured papules on her knees and some thickening of the adjacent skin. Over the next 2 months her hands and elbows were similarly affected.

She attended the out-patient clinic regularly and her bowel disease was kept under good control. However, in January 1975, her Crohn's disease flared up and she felt generally ill with nausea and vomiting. She was admitted to the hospital and found to be in renal failure. Investigations pointed to an obstructive aetiology and a laparotomy was undertaken which revealed a large cystic tumour in the right ovary. This was compressing both the ureters and there were secondary deposits on the pelvic wall and in the liver.

Examination (December 1974). Her skin rash consisted of pale, closely set dermal papules over the dorsa of the hands, elbows, shoulders and knees, suggesting a diagnosis of lichen myxoedematosus. A general examination otherwise was unremarkable except for scars from previous surgery.

Investigations. Her ESR has varied between 30 and 70 mm/h. Her blood urea was first found to be raised at 127 mg% in January 1975. This reached a peak at 222 mg% but started to fall rapidly when the ureteric obstruction was relieved. Her serum thyroxine and free thyroxine index are at the upper limit of normal and Bence Jones proteins were never detected. Serum protein electrophoresis in 1972 showed an increase of beta and gamma globulin fractions. This pattern has persisted and immunoelectrophoresis in December 1974 showed a small but distinct abnormal band in the late gamma globulin zone. A quantitative estimation of the immunoglobulins gave the following results: abnormal monoclonal IgG 500 mg/100 ml, normal polyclonal IgG 1500 mg/100 ml (normal 700–1700 mg/100 ml), IgA 180 mg/100 ml (normal 25–425 mg/100 ml) and IgM 57 mg/100 ml (normal 47–170 mg/100 ml). A repeat estimation after removal of the ovarian cyst has not shown any change.

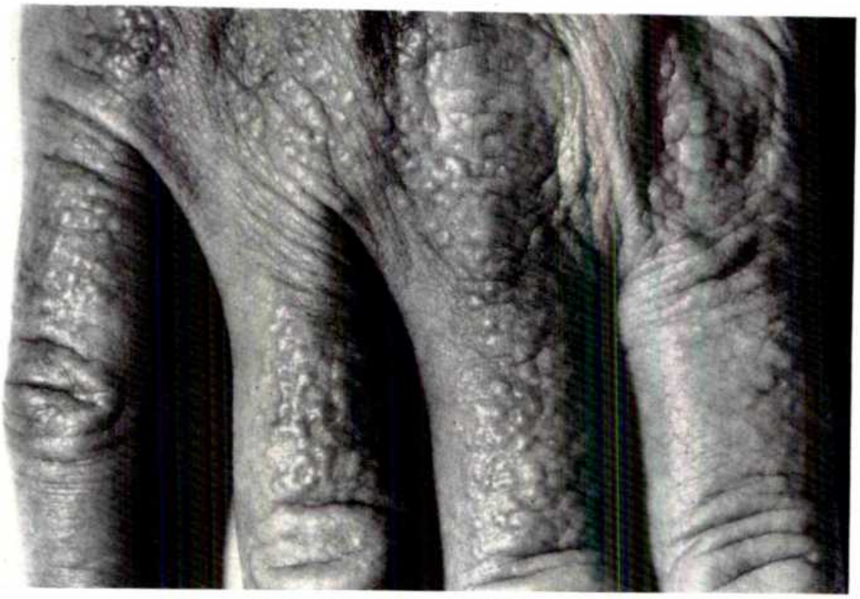

FIGURE 1. Lichen myxoedematosus lesions on fingers.

Sternal marrow examination showed an increased number of plasma cells but there was no other abnormality.

Skin biopsy (reported by Professor Wilson Jones) 'There is a dispersed infiltrate of histiocytic cells or fibroblasts in the dermis, associated with some increase in connective tissue mucin. I think this could well be lichen myxoedematosus and could be the rather more sclerotic stage in the process, in which mucin accumulation is not a marked feature'.

Histology of the ovarian tumour was that of a mucinous cystadenocarcinoma. Rectal biopsy showed mild changes suggestive of ulcerative colitis rather than Crohn's disease.

Barium enema favoured the diagnosis of Crohn's disease. It was normal in 1961 but when repeated in 1972 showed loss of haustrations, narrowing and deep ulcers in the entire length of the colon.

Management. Her Crohn's disease ran an uneventful course and could be controlled with prednisolone (10 mg daily). Azathioprine was tried in 1973 but had to be stopped because of intolerance. In January 1975, however, an exploratory laparotomy revealed a malignant ovarian cyst which was removed. The other ovary, uterus and both fallopian tubes were also removed. The ureters had to be re-implanted (uretero-ureterostomy). She is currently receiving chemotherapy for the metastatic deposits. Her skin rash seems to have improved somewhat without any specific therapy.

Comment. It is indeed remarkable that this woman has lived through five major diseases including two carcinomas, thyrotoxicosis, comedo carcinoma of the breast, Crohn's disease, lichen myxoedematosus and mucinous carcinoma of the ovary. There is no obvious statistical or aetiological connection between these diseases. There is, however, one feature in common between mucinous ovarian carcinoma and lichen myxoedematosus and that is deposition of mucin. This is also seen in thyrotoxicosis, though not in every case, as pretibial myxoedema. This last feature was not seen in the case presented.

(4) Scleromyxoedema

DR RAJ KUBBA AND DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge
Previously shown at The Royal Society of Medicine—(Rook & Moffatt, 1956)

Mr F.L., aged 63 years. Butcher.

History. In June 1955, he first presented with soreness of his fingers and swelling and erythema of both hands. These were followed by the gradual appearance of pale papules on his forearms, face, ears, neck and buttocks. A diagnosis of lichen myxoedematosus was then made and confirmed by histology. In 1964 he developed mottled erythema below both scapulae and around his umbilicus. By January 1957 his papular eruption had cleared but recurred in October 1973.

Since 1958 he has been developing progressive tightness and thickening of the skin, most marked over his hands and face. By 1964 he had developed severe sclerodactyly and so much tightness of the mouth that he was unable to take out his dentures. He was investigated extensively at that stage but no systemic involvement was detected. He has complained of widespread aches and pains in his joints as well as in his muscles, since the early part of his illness. In 1966 he experienced pain and weakness in his forearms and thighs. Neurological investigations produced equivocal evidence of polymyositis. He improved rapidly when given prednisolone (30 mgs daily) but promptly relapsed when this was withdrawn in 1969. He has since been on a small maintenance dose.

Hypertension was discovered in 1969, with signs of left ventricular hypertrophy. He had episodes of paroxysmal nocturnal dyspnoea, left ventricular failure and ascites during 1972.

He also has chronic renal failure which first became evident in 1969. He has never had proteinuria.

Examination (February 1975). He has sclerodactyly, and marked sclerosis and telangiectasia of the face. His blood pressure is 160/85 mmHg. There are no other abnormal physical signs.

Investigations. Abnormal. Protein electrophoresis showed increased gamma globulins on several occasions; immunoelectrophoresis showed abnormal monoclonal IgG. Quantitative estimation showed IgA <50 mg/100 ml (normal 125–425), IgM <30 mg/100 ml (normal 47–140), abnormal monoclonal IgG 900 mg/100 ml, normal polyclonal IgG 550 mg/100 ml (normal 700–1700). Blood

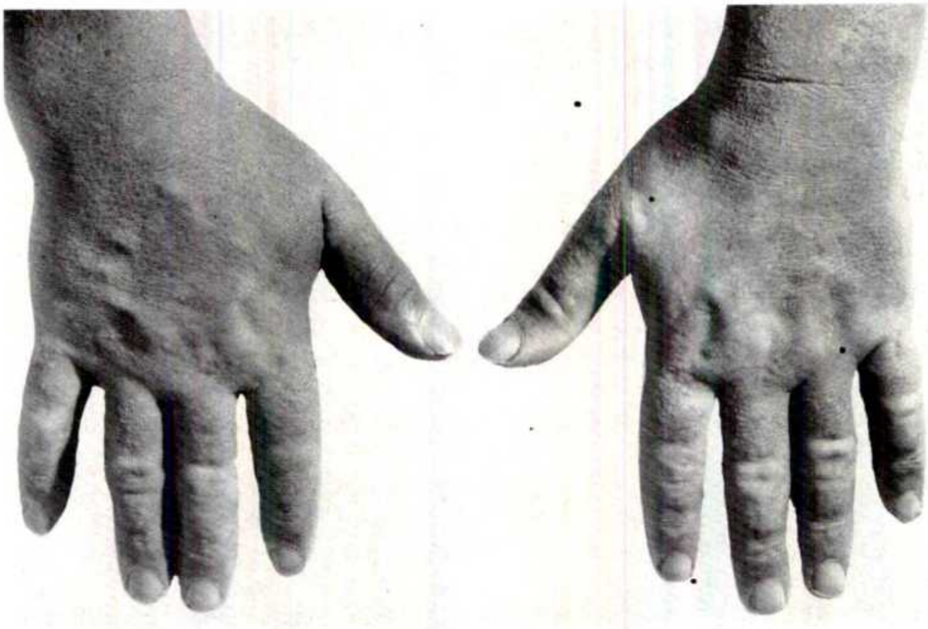

FIGURE 1. Lichen myxoedematosus in 1956.

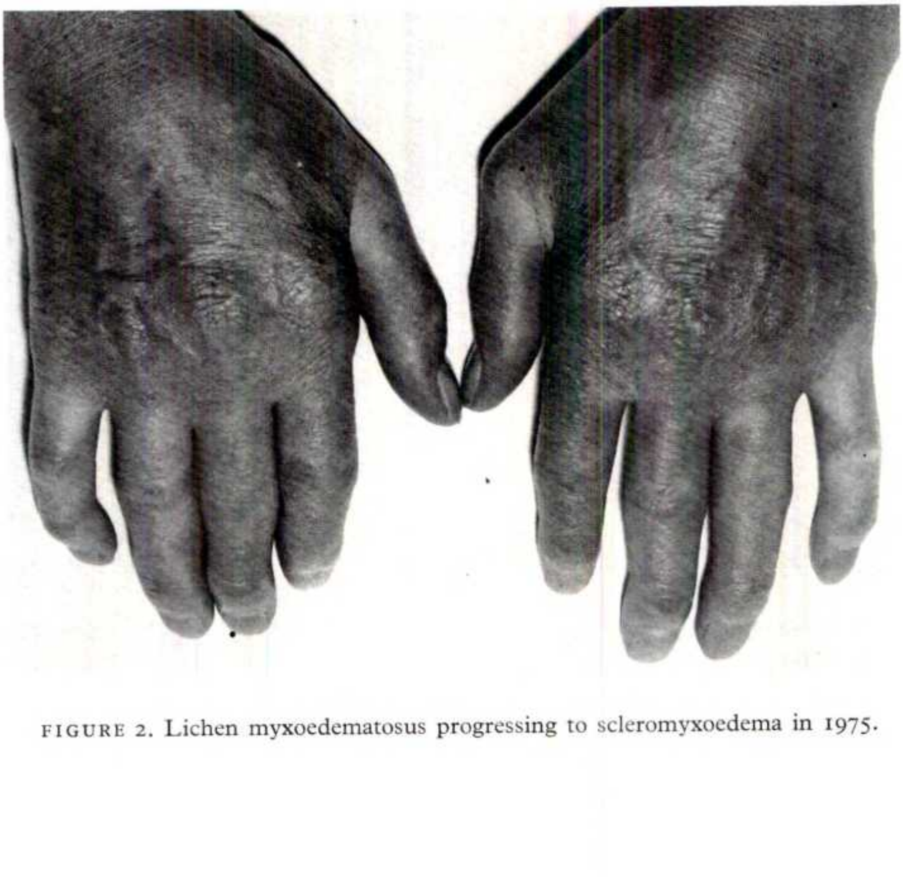

FIGURE 2. Lichen myxoedematosus progressing to scleromyxoedema in 1975.

urea 50–180 mg% (usually around 80 mg%), creatinine clearance 34 ml in the 1st h (102 ml in 1964).

X-rays of both hands showed early arthritis in the distal interphalangeal joints and both wrists. Barium enema showed diverticulosis.

EMG showed polyphasic units and some 'dive-bombing' noises for up to 5 s.

Histology. In 1955, a skin biopsy from the dorsal surface of a finger showed only interstitial infiltration of the upper corium with a metachromatic substance, which was identified as mucin by special stains. In 1964, a biopsy from an erythematous patch on the anterior abdominal wall also showed moderate deposits of mucin in the upper corium.

Muscle biopsy (left-deltoid) showed mild, non-specific degenerative changes.

Normal or negative. Full blood count, E.S.R.; serum electrolytes, calcium, phosphorus, liver function tests, cholesterol, blood glucose, W.R., Paul Bunnell test, creatinine phosphokinase; L.E. cells, autoantibodies and Rose Waaler tests; urinary amino acids, 17 ketosteroids and 17 ketogenic steroids.

X-rays of chest, shoulders and hips. Barium swallow, intravenous pyelogram and electroencephalogram.

Treatment. In 1974 he was treated with intermittent courses of melphalan, and later on a continuous regimen. A total of 335 mg of melphalan was given between 5.7.74 and 3.12.74. Improvement first became evident on 23.1.75 and by 27.2.75. his lichen myxoedematosus had cleared. The sclerosis of the skin was, however, unaffected.

He is also receiving enteric coated prednisolone, 7·5 mg daily, methyl dopa, spironolactone, frusemide and Slow K.

Comment. Melphalan has previously been used with success in the treatment of lichen myxoedematosus (Feldman *et al.*, 1969). Our case also demonstrates that spontaneous resolution can occur. Other interesting features of this case are unexplained chronic renal failure and a polymyositis. An association of scleromyxoedema and dermatomyositis was recently reported (Johnson *et al.*, 1973).

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(5) Discoid lupus erythematosus

DR M.A.SMITH

Essex County Hospital, Colchester

Mrs E.M., aged 48 years. Housewife. Hospital case no. U.3 47 59.

History and examination. In 1953 she developed patches of lupus erythematosus on the right side of her nose and cheeks.

In 1966 the lesion on her nose was grafted with split skin from behind her ear. Other patches were helped by infiltration with triamcinolone suspension.

In 1969, LE lesions appeared in her nostrils and on her cheeks and ears. In 1971 she developed malaise in the summer. She was treated with chloroquine 200 mg twice daily for 4 months, and again

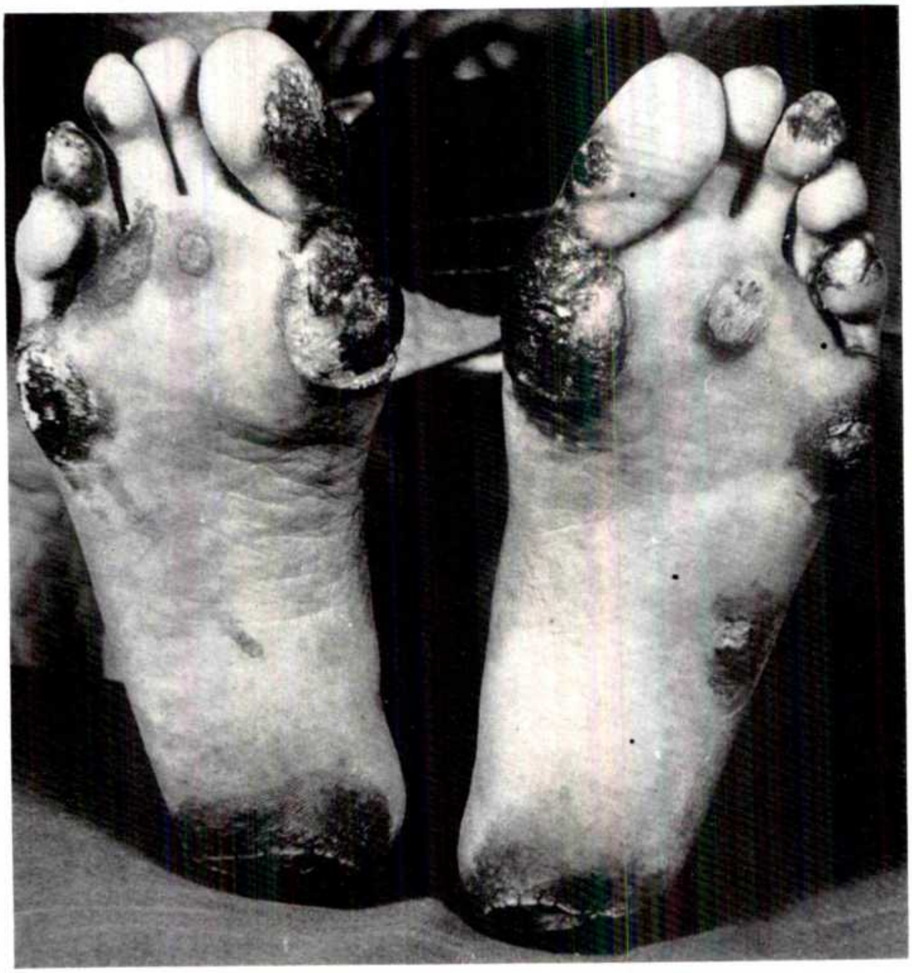

FIGURE 1. Lupus erythematosus on soles.

for 5 months in 1972. It was not given in April 1973 owing to the development in the right eye of a minimal blind spot which had disappeared by June. Later in 1973 she developed LE on the buccal mucosa, deltoid areas, upper back, and flexor aspects of the palms, fingers, soles and toes. She also developed malaise, arthralgia, low grade pyrexia and loss of weight.

Prednisolone, 20 mg daily, and azathioprine, 50 mg twice daily, were given for a few weeks, with little improvement, and in August 1973 she was admitted to St George's Hospital, London. Coal tar paste occlusive bandages were applied to the hands and feet, and later 10 ml of triamcinolone suspension was infiltrated into the hands and feet under a general anaesthetic. Mepacrine provoked a lichenoid eruption, and in May 1974, when her malaise and skin lesions were worse, prednisolone, 20 mg daily, caused some improvement.

In January 1975 she had a blood pressure of 190/96 mmHg, a tachycardia, Cushingoid appearance, malaise, and swelling of the salivary glands, not apparently controlled by the steroids so prednisolone was discontinued. Since then the only systemic treatment has been Distalgesic, indomethacin, frusemide and Slow K.

Investigations. Tests for LE cells have always been negative. The test for anti-nuclear factor became weakly positive in the last 2 years, but has otherwise been negative.

There has been a tendency to leukopenia (around 3000 per mm^3); ESR 10–20 mm in 1 h.

In 1971, thyroglobulin, mitochondrial, smooth muscle and gastric parietal cell antibodies were not demonstrable. Radio-isotope studies have shown that she is euthyroid.

Comment. In the past 2 years this patient has been crippled by the lesions on her hands and feet; the other skin and mucosal lesions have been quiescent. From time to time she gets systemic symptoms of malaise. Grafting for some of the lesions on the feet is under consideration.

(6) Juvenile generalized pustular psoriasis

Dr J.B.Lyon

Ipswich Hospital

P.J., male, aged 5 years. Hospital case no. 53 59 18.

History and examination. Since May 1973 he has had episodic attacks lasting up to 2 months in which his skin becomes erythrodermic and studded with numerous sterile pustules, as well as showing scaling. During the attacks he is toxic and ill, with high septicaemia-like temperature and rigors.

Family history. His parents and siblings have no history of psoriasis, but his paternal aunt has.

Investigations. Histology of a pustule confirmed the diagnosis of pustular psoriasis. Hb 8 g%, WBC up to 35,000/mm^3, with 80% polymorphs, ESR 46 mm in 1 h. Serum calcium 8·6 mg%; blood urea and liver function tests normal. Blood cultures, sterile. Swabs from pustules have been sterile, but from his skin surface have grown *Staph. aureus* and *Staph. albus.* Immunoglobulins IgA, and IgG normal; IgM low (41 mg%). Alpha-2 globulin is raised.

Treatment. During the first attack prednisone 5 mg twice daily and penicillin were of no benefit. Methotrexate has been used in subsequent attacks, which occur every 2 to 4 months. Usually three or four courses of 1·25 mg daily for 4 days followed by a break for 3 days are needed to bring an attack under control. He also receives Floxapen, iron and nystatin for oral candidiasis. Local applications have been bland, non-steroid preparations.

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(7) Nummular eczema with staphylococcal sensitivity

Dr Esther Welbourn, Dr R.H.Champion and Dr W.E.Parish*

Addenbrooke's Hospital, Cambridge, and The Lister Institute, Elstree (now at Sharnbrook, Bedford)*

Mrs H.G., aged 64 years. Clerk.

History. She gives a 36 year history of nummular eczema, initially intermittent but for some years almost unremitting. Individual exacerbations have sometimes been associated with depression. She has also had frequent episodes of staphylococcal boils and styes. These, and other such infections as tonsillitis and dental sepsis, as well as complicating attacks have often aggravated and even initiated exacerbations of the eczema.

In 1970 she developed a clinical nickel sensitivity, confirmed by patch testing.

Family history. None of her parents or her sixteen siblings have had eczema or atopic disorders.

Examination. She has a typical nummular eczema mainly on extensor aspects of her limbs.

Investigations (Demonstration available). Repeated swabs, not surprisingly, have grown *Staph. aureus* from eczematous lesions as well as from normal skin, nose and infected lesions.

Intradermal tests have repeatedly shown an immediate, a 4 h (Arthus) and a delayed type hypersensitivity to various *staphylococcus aureus* (pyogenes) antigenic preparations, and, to a lesser extent to *Staphylococcus albus* preparations. A skin sample of an Arthus response showed slight neutrophil infiltration, and by immunofluorescence, staphylococcal antigen, IgG and complement C3. No IgE to account for the immediate response was seen in the skin sample.

In agglutination tests using red cells treated with phenol extracted staphylococcal antigens including teichoic acid, the direct titre was 1/32, the antiglobulin titre for IgG was 1256 and for IgM nil (i.e. <32). The complement lysis titre was 1/16. When total extracted soluble antigens or protein antigen were examined by similar methods, the direct titre was 1/128, and the antiglobulin titre for IgG was 1/1000. Complement was fixed at a serum titre of 1/4. The serum therefore contained IgG antibodies to several staphylococcal antigens, and the complexes fixed complement weakly. It was demonstrated that the patient's IgG was a true antibody, with binding of the Fab portion, and not an antigen non-specific effect of the Fc portion of the molecule.

Monolayers of human embryo cells, or homologous skin cells, firmly absorbed staphylococcal antigens added to the cultures. Subsequent treatment with the patient's antibody to the antigens, plus complement, lysed or severely damaged the cells.

Comment. This patient is one of 122 patients with disseminated eczema who have been investigated clinically and immunologically for the role of bacterial, especially staphylococcal, sensitivity. The results will be published shortly.

She and other patients provide circumstantial evidence that various types of allergic reaction to various staphylococcal antigens may play an important contributory, but not necessarily initiating, role in a considerable proportion of these patients. The therapeutic response to topical and systemic antibiotics is often encouraging, but not as dramatically so as might be wished.

(8) Nickel sensitivity resembling bullous pemphigoid

DR RAJ KUBBA AND DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge

Mrs C.B., aged 55 years, housewife. Hospital case no. 44 59 07.

Past history. In 1962 she had a Pott's fracture of her left ankle, which was fixed with a Vitallium screw. In 1966 she developed hand eczema which took several months to clear.

Present illness. This began in June 1974, when she started to develop blisters on her left leg. These continued to appear, a few at a time and caused only minimal discomfort. On direct questioning she admitted to having reacted to metals many years ago.

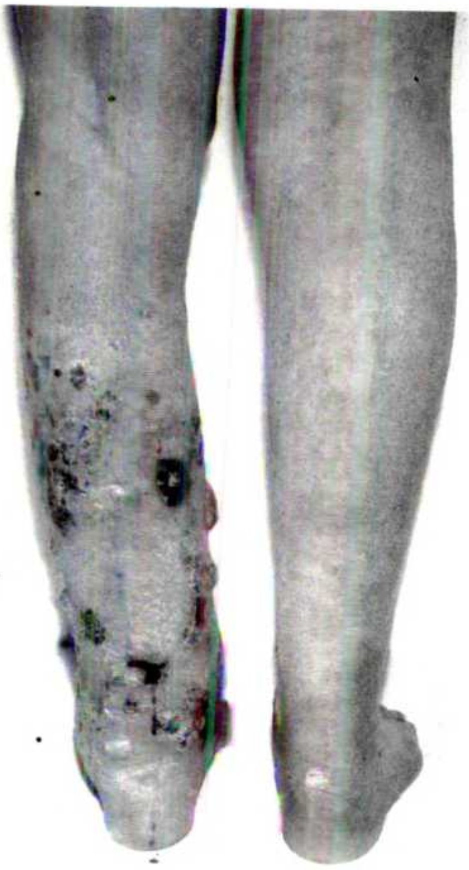

FIGURE 1. Pemphigoid-like eruption on left leg.

Examination (June 1974). She is quite a fit woman, though partially deaf. She had a few intact blisters on her left leg, below the knee. They were tense with clear serous contents and measured 1–4 cm in diameter. There were several other burst blisters in various stages of healing. There was no eczema on the affected leg and no scarring. There was a solitary patch of discoid eczema above her right elbow.

Investigations. Patch tests: nickel sulphate + + (eczematous reaction), cobalt chloride + +.

Intradermal tests: nickel sulphate (0·05 ml, 0·01%) caused 8 mm weal at 15 min, 20 mm weal at 12 h, and a tense bulla by 48 h. There was no significant reaction to control, cobalt, chrome, molybdenum, candida albicans, trichophytin or tuberculin.

A biopsy from an intact bulla on the left leg was consistent with a diagnosis of bullous pemphigoid. A biopsy from the bullous intradermal reaction also showed a subepidermal bulla and an acute inflammatory infiltrate consisting of polymorphonuclear neutrophils and eosinophils.

Immunofluorescence: (Dr K. Kraft). Both biopsies and the patient's serum were examined for direct and indirect immunofluorescence respectively. No immunofluorescence could be seen anywhere, particular attention being given to the basement membrane zone.

X-ray of her left ankle showed the presence of a screw and secondary osteoarthritis.

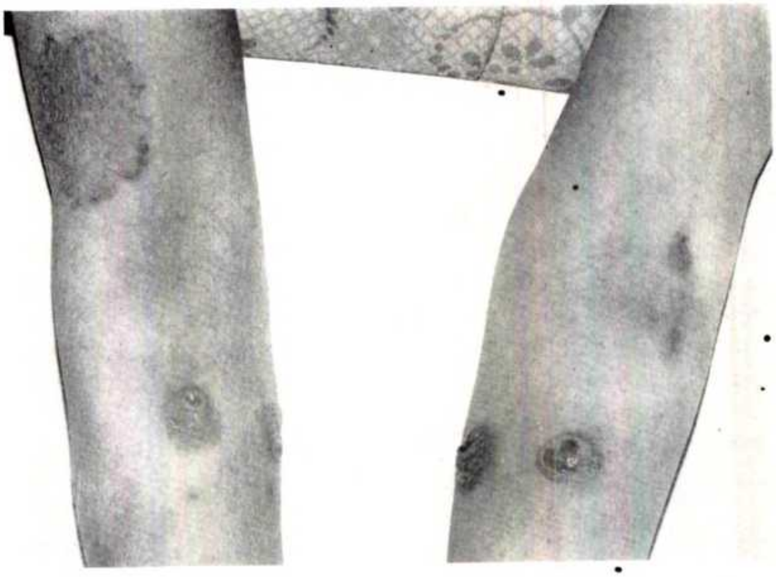

FIGURE 2. Bullous reaction to intradermal nickel injections at 48 h. Plaque of eczema on right upper arm.

Treatment. Her bullous eruption could be suppressed by using Dermovate ointment under polythene occlusion. The metallic screw was removed on 13.3.75.

Comment. There is evidence to suggest that her pemphigoid-like eruption was caused by nickel leaching out of her metallic screw. There are several reports in the literature of cutaneous reactions to metallic implants. Such reactions are usually eczematous, an exception being a case of an urticarial reaction to a Vitallium nail (McKenzie, Aitken & Ridsdill-Smith, 1967). More recently metal sensitivity has been shown to be responsible for loosening of joint prostheses (Mervyn-Evans *et al.*, 1974).

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(9) Morphoea following chicken pox

Dr J.Singh and Dr G.A.Beck

Peterborough District Hospital

K.T., female, aged 12 years. Hospital case no. 21 23 09.

History. This patient had an unexceptional attack of chicken pox 4 years ago and following this developed a number of whitish patches. Recently these have increased in size. The lesions do not itch or hurt.

Family history. There is no significant family history.

On examination. There are eight lesions distributed over her back, abdomen and upper thighs varying in size from 0·5 to 2·5 cm in diameter. Each lesion is surrounded by a narrow pink, scaly margin and centrally there are prominent follicular orifices. The lesions feel hard and are mobile.

Investigations. Her ESR is 5 mm/h and her blood count is within normal limits.

Histology. The thigh skin biopsy shows marked eosinophilic degeneration of the collagen within the dermis and there is a patchy lymphocytic infiltrate in the lower dermis extending into the subcutaneous tissue. There is hyperkeratosis of the overlying epidermis but no other features.

These changes are consistent with those seen in morphoea (F. J. Fawcett).

Comment. The lesions have remained unchanged over the past 4 years. The patient's mother says that they have increased in size but the patient herself feels that they are about the same size. No treatment has been given.

We are not aware of this cutaneous complication of chicken pox.

(10) Morphoea arising in a hypoplastic breast

DR DAVID GOLDIN AND DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge

R.S., female, aged 26 years. Hospital case no. 42 09 19.

History. Two years ago a small red spot appeared on her right breast, which has been hypoplastic since puberty. This rapidly enlarged and became indurated over a period of 6 months.

On examination. There was a plaque of morphoea arising just below the right nipple and extending to the mid-line in a linear fashion. It was fixed to the underlying bones.

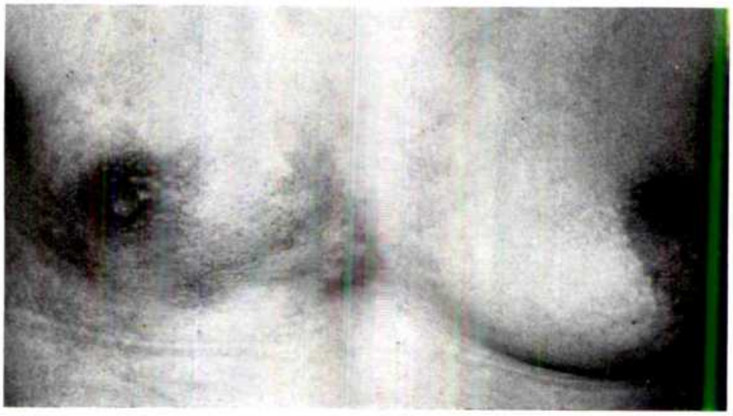

FIGURE 1. Morphoea involving hypoplastic breast.

Investigations. Skin biopsy confirmed the diagnosis of morphoea. There was no evidence of malignancy. Chest X-rays revealed no other abnormality.

Progress. There has been some regression. The morphoea is now only fixed to the sternum. Haelan Cream controls the irritation.

(11) Calcinosis universalis

DR RAJ KUBBA AND DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge

Miss C.M., aged 35 years. Hospital case no. 33 82 17.

History. From the age of 3 years she has had progressive calcification in the subcutaneous tissues and muscles, with contractures of her limbs. There is no positive evidence that it started as dermatomyositis. She was unable to walk before she was 8, and has been in a kneeling position since the age

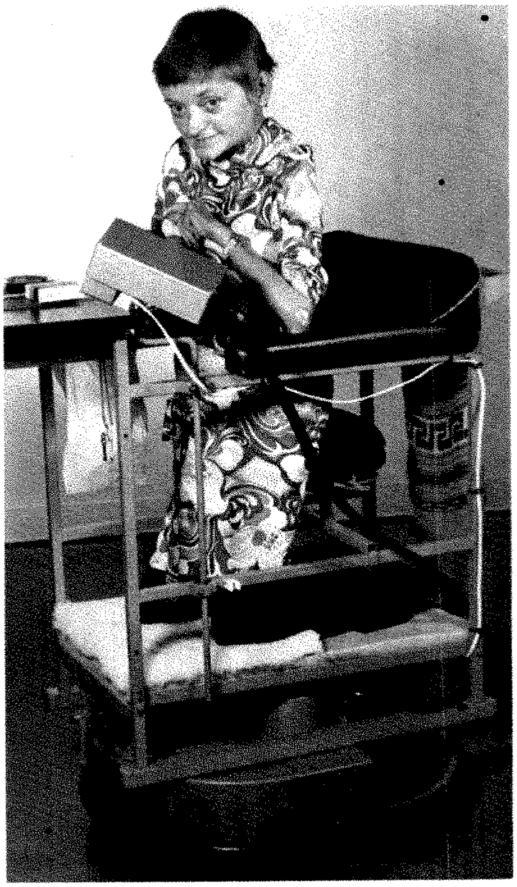

FIGURE 1. Calcinosis universalis. The patient in her specially designed mobile chair.

of 13 years. Calcium deposits are more pronounced around the pelvic and shoulder girdles and elbows and knees. They often get infected when calcium is extruded by ulceration and sinus formation, followed by slow healing.

Family history. Both her parents died of unrelated illness. She has one brother who is healthy.

Examination. She is intelligent and mentally alert but grossly underweight. There is gross calcification of subcutaneous tissues and muscles, with severe contractures of her hands, arms and legs. Her skin is shiny and atrophic, and there are multiple discharging sinuses with calcium deposits at the base. Her locomotor function is limited to movement of two fingers of the left hand and some movement at elbows and shoulders. There are no abnormalities of respiratory, cardiovascular or central nervous systems.

Investigations. She has had extensive and repeated investigations at various centres. These have shown that deposits are made up of calcium phosphate and are not in equilibrium with the extracellular fluid. Skeletal surveys have shown varying degrees of soft tissue calcification. The following tests were normal or negative: serum calcium and phosphorous, creatine phosphokinase, plasma proteins, liver and renal function tests, Rose Waaler test and electromyography.

Treatment. Her drug therapy has included corticosteroids, ACTH, EDTA, Aludrox, and probenecid. More recently she was treated with diphosphonates (sodium etidronate 600 mg daily) for 1 year. During this period her serum calcium, phosphorus and urinary hydroxyproline were monitored and skeletal surveys carried out at 6-monthly intervals. There was no clinical or radiological improvement.

Social Background. She has always needed a great deal of care and attention. After the death of her father in 1969, with no other available relatives or friends, this could only be provided in hospital. She spent 3 years in our dermatology ward. No suitable accommodation in a young chronic sick unit was found. For the past year, largely through her own determination and with great help from the Social Services and from Voluntary aid, she has managed to live by herself in an adapted council house. She needs help with turning over, moving, dressing and feeding. Someone in the flat above her helps her at night. She has a specially designed mobile chair and 'Possum' (POSM—patient operated selector mechanism). With a switch like a morse code key she can switch on her television or her record player, open and close the front door, and several other things. She also has a Possum typewriter.

She has not only managed to obtain an external honours degree in philosophy and theology from London University but has also been able to write children's stories for the B.B.C. and to review children's books for the local newspapers.

(12) Urticaria, amyloidosis and deafness. Muckle Wells syndrome

DR R.H. CHAMPION

Addenbrooke's Hospital, Cambridge

Previously shown at St John's Hospital Dermatology Society in December 1962. Eastern Counties Dermatological Society in March 1972

A.W., female, aged 29 years. Hospital case no. 20 90 30.
S.W., female, aged 4 years.
S.W., male, aged 1 year.

History and examination. Mrs W. has had a rash every day of her life from the age of 2 years. Individual lesions last for up to 24 h, and sometimes itch. The eruption consists of erythematous macules, some of which are maculopapular or even urticarial, up to 2 cm across (Fig. 1). The lesions

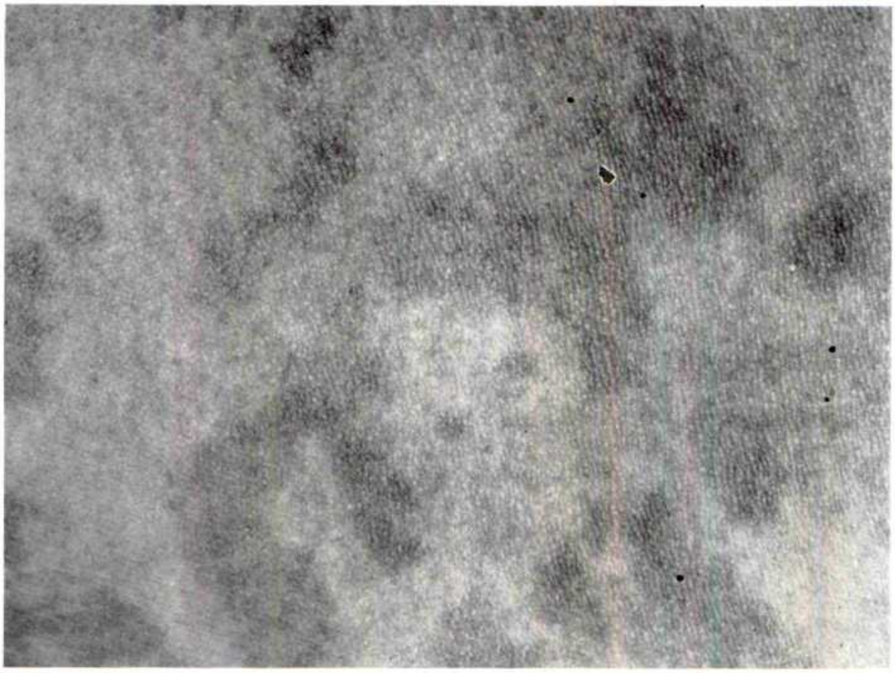

FIGURE 1. Close-up of irregularly shaped erythematous and urticarial lesions.

are often irregularly shaped and confluent. Other clinical features have included: joint pains of moderate severity from the age of 2 years ('atypical rheumatic fever') but without true arthritis; red sore eyes due to episcleritis and vernal conjunctivitis; anaemia; perceptive deafness from adolescence, worse recently.

The children. Both children were noted to have an exactly comparable rash from the day of birth. With a daily changing pattern this rash has never cleared completely.

The daughter, now aged 4 years, has had rather ill-defined joint pains in her knees and ankles for the past year, and a doubtfully abnormal audiogram. Otherwise both children are well. There are no other siblings.

The parents of Mrs W. Both her parents are well clinically, and have normal blood counts and serum proteins. She has one brother who is unaffected.

Investigations. Mrs A.W. Abnormal. Hb 7–11 g % (from early childhood); normochromic anaemia, unresponsive to oral and parenteral iron. WBC 14–22,000/mm^3, polymorph leukocytosis. ESR 80–110 mm/h (from early childhood). Serum proteins 8·5 G%, raised Gamma, Beta and Alpha-2. Serum iron 39–65 μg/100 ml.

Normal or negative. X-rays of chest, knees and hands; blood urea; urinalysis; LE cells, anti-nuclear factor, anti DNA antibodies; serum complement; WR, toxoplasma, rubella, brucella and Australia antigen serology; electron microscopy of centrifuged serum for virus or immune complex particles. Renal biopsy has not been performed.

The children

Hb 8·3 g%. WBC 20,000 mm^3 (46% polymorphs).
Hb 10·0 g%. WBC 14,000 mm^3 (46% polymorphs).

Comment. This syndrome was originally described from Derbyshire in 1962 by Muckle & Wells. It seems that seven other families or isolated cases have been recorded since (Black, 1969; Lagrue *et al.*, 1972). The characteristic feature is the skin eruption but this has been absent in some familial cases. It is erythematous, sometimes urticarial and because of the small lesions may have a superficial resemblance to cholinergic urticaria from which it clearly differs. The eruption closely resembles that of Still's disease, under which diagnosis this family was previously described (Jones & Champion, 1973). Onset is usually in childhood but may be later. Onset at birth in these two children is remarkable. Perceptive deafness with nerve and cochlear degeneration often starts in later childhood, but may be absent. Renal amyloidosis and renal failure tend to occur in adult life, but not in all cases. The relationship of this disease to other types of hereditary primary amyloidosis without urticaria, and to familial Mediterranean fever is obscure (Gafni, Sohar & Heller, 1964; Cohen, 1967).

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(13) Follicular keratoses

DR DAVID GOLDIN AND DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge

W.F., male, aged 72 years. Retired. Hospital case no. 44 67 98.

History. In 1947, when he was aged 45 years, he developed follicular keratoses. Although the serum vitamin A level was normal, he was treated with oral vitamin A and resolution of the rash occurred after 6 months. He reports that his skin remained clear until the summer of 1974, when the rash recurred, starting on his shoulders and spreading to the body. Six months before the recurrence, he was found to have hypertension, which is controlled on methyl dopa.

Family history. There is no relevant family history.

Examination. There is a patchy distribution of follicular keratotic papules on his shoulders, back and the lateral aspects of the thighs.

Investigations. Full blood count, ESR, liver function tests and chest X-ray are normal.

Histopathology of skin taken from the thigh shows keratin-plugged, dilated follicles which are surrounded by and superficially invaded by a moderate cellular infiltrate, principally composed of lymphocytes.

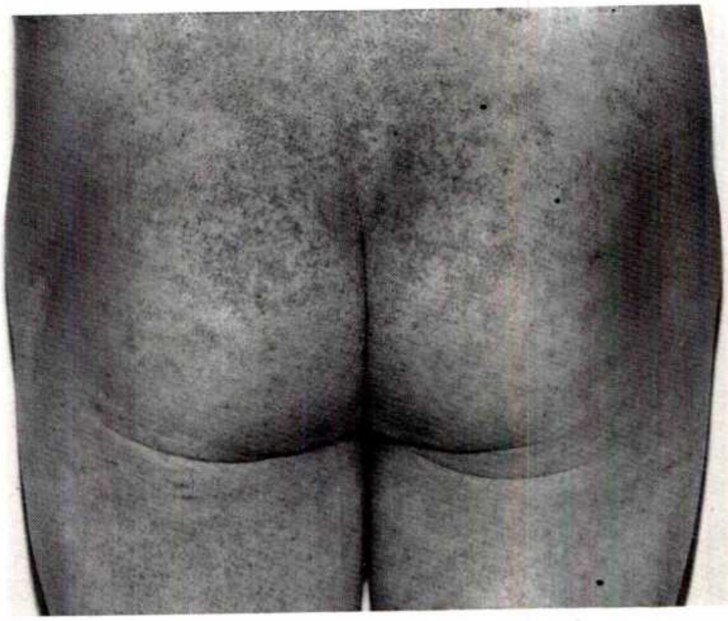

FIGURE 1. Follicular keratoses on buttocks.

Treatment. Retinoic acid cream was helpful initially, but was discontinued because it made him sore. He is now using 2% salicylic acid ointment.

Comment. This case of follicular keratosis does not appear to fit into any of the named entities.

(14) Folliculitis

Dr J.Singh and Dr G.A.Beck

Peterborough District Hospital

B.T., female, aged 26 years. Library assistant. Hospital case no. 16 39 35.

History. For 4 years this patient has had a papular eruption confined mainly to her arms, breasts and thighs. Individual spots fade in a few days leaving a stain. The lesions start as small whiteheads, or sometimes as yellow heads, but dry up to leave brown dots that can be picked out. The lesions do not itch or hurt.

Past history. In December, 1971 she presented with ovarian endometriosis. Two cysts, one from each ovary, were removed. Both ovaries were left behind. She relates the start of the spots to this operation.

Family history. Her father has eczema and she herself gets occasional hay fever.

On examination. Most of the lesions are tiny follicular scabs distributed over her upper arms, breasts and inner aspect of thighs. There is also extensive excoriation of the lesions.

Investigations. Her ESR is 7 mm/h and her blood count and serum electrolytes are normal. Her

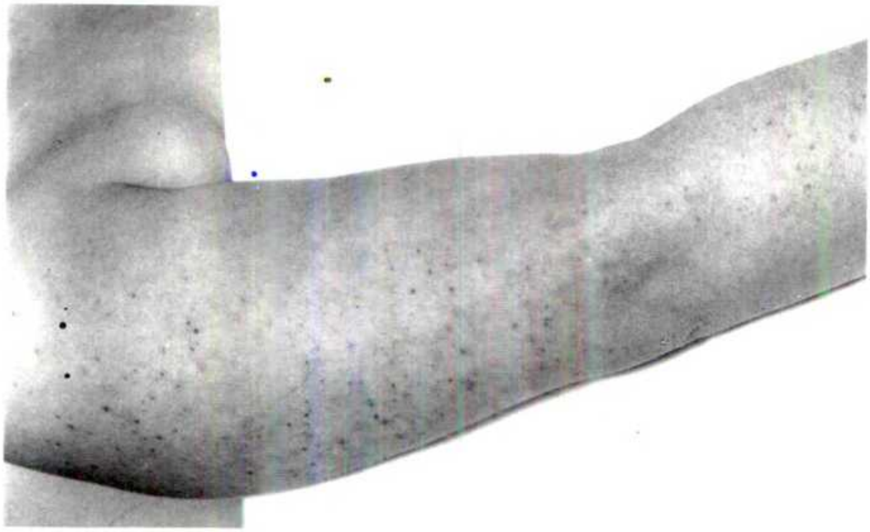

FIGURE 1. Follicular lesions on arm.

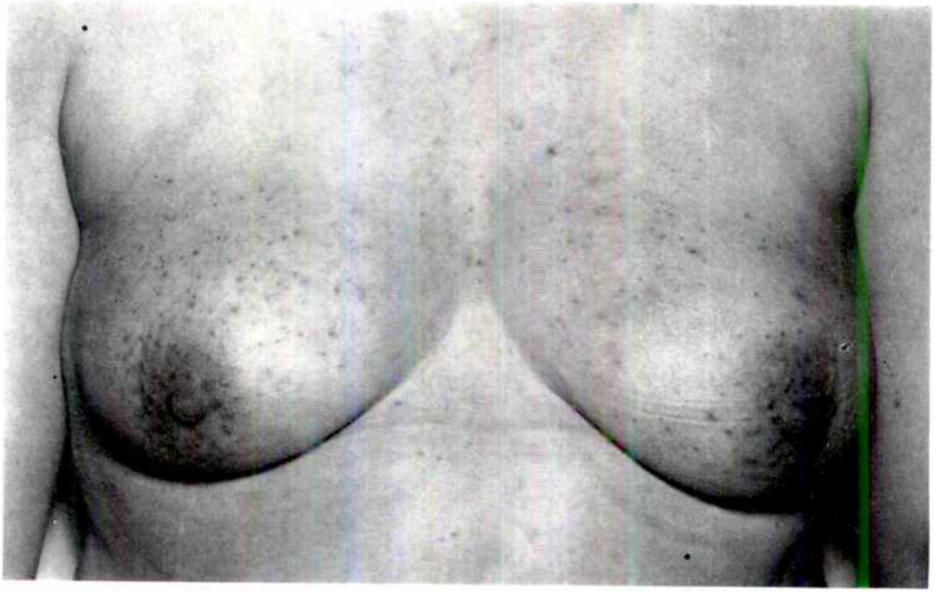

FIGURE 2. Follicular lesions on breasts.

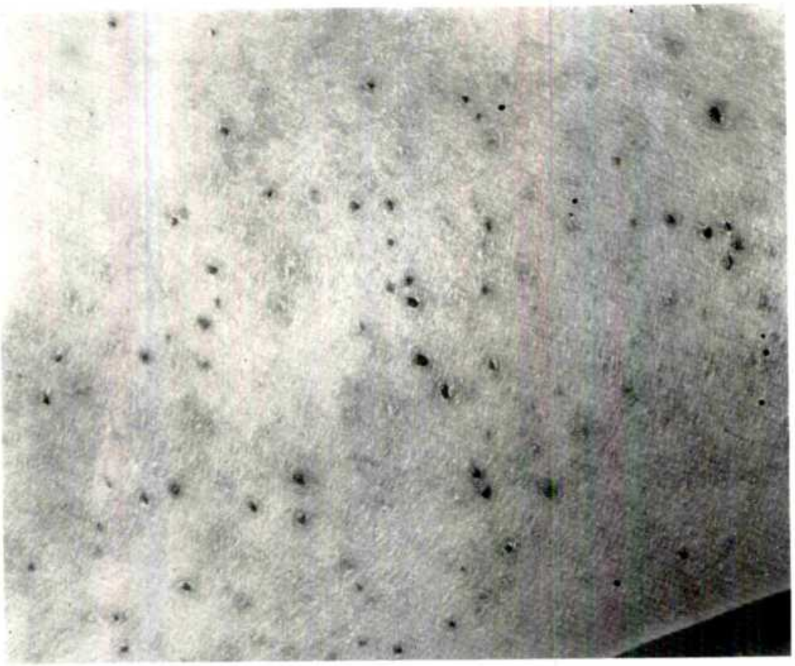

FIGURE 3. Close-up of lesions on arm.

chest X-ray is normal. Mycological and bacteriological investigations have been negative. *C. Minutissimum* has been isolated from her toe webs. The Mantoux Test is negative (1:10,000). The auto-antibody screen is also negative and her immunoglobulins are within normal limits.

Histology. Sections show hyperkeratosis and a small area of parakeratosis. There is marked plugging of the hair follicles by keratin and one biopsy shows within the epidermis a small superficial abscess composed of neutrophil polymorphs within the edge of a hair follicle. Within the dermis there is a mild patchy infiltrate, of lymphocytes and plasma cells, some of which is arranged around small blood vessels.

These are the features of a non-specific folliculitis (F.J.Fawcett).

Course and treatment. Initially Terracortril spray did not improve her skin and she was given tetracycline, 250 mg, daily, for 8 months. During this period two courses of ultra-violet light were also given. As there was no improvement retinoic acid lotion (Retin-A) was tried without success.

Comment. As this patient has been in the habit of having two or three baths a week, always with added bath oil, an initial diagnosis of 'bath oil acne' was made. She has stopped using the bath oil for the past 1½ years, but the lesions have not changed much during this period.

(15) Follicular pityriasis alba

DR G.A.BECK
North Cambridgeshire Hospital, Wisbech

L.W., female, aged 8 years.

History. For 3 years she has had gradually spreading patches on her lower limbs. These started on

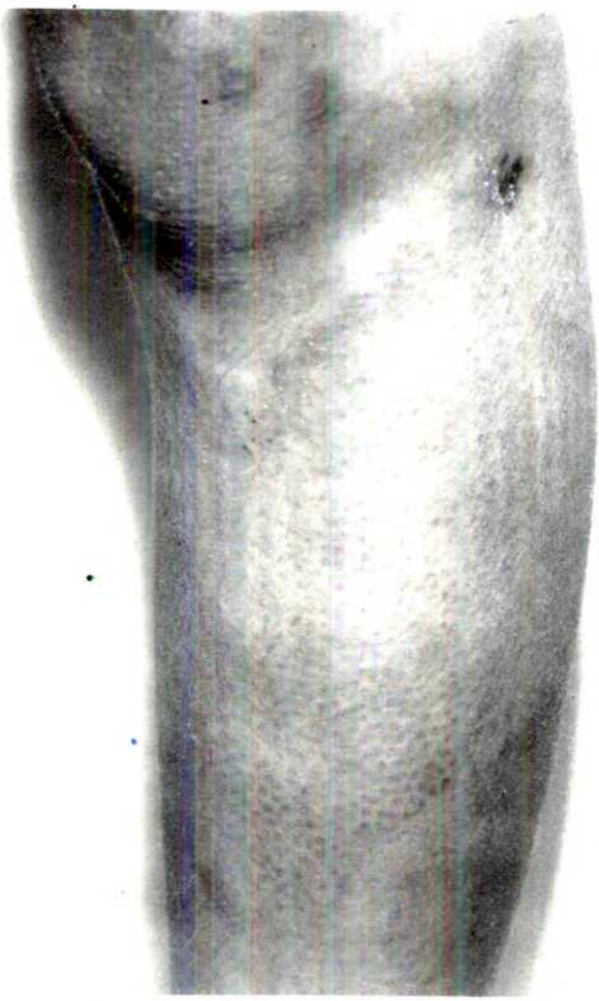

FIGURE 1. Plaque of follicular lesions on leg.

her left leg, then her right thigh, and during 2 years of observation she has gradually developed fresh smaller patches on both legs and the left thigh, all on the anterior surface. For the past year she has had one patch on her right forearm.

Examination. The lesions consist of well defined patches of paler skin studded with prominent follicles which tend to be rather more marked towards the margins. There was faint erythema when she was first seen, but this has never been marked. The original lesion on her left leg is still the largest ($12<9$ cm), others varying with duration down to about 1 cm.

Investigations. Mycology has been negative on three occasions (Dr Clayton). Blood count normal, ESR 4 mm/h.

Histology. 18.5.73. (Dr D.Eakins) 'Slight hyperkeratosis and parakeratosis. Some fibrosis round hair follicles. Few inflammatory cells mainly lymphocytic in the dermis. Features do not permit a diagnosis of follicular mucinosis'.

17.12.74. (Professor E.Wilson Jones) 'Mild chronic eczema with some degree of serous crusting and parakeratosis. At one point the reaction seems to be centred at the mouth of a follicle. Changes too inflammatory for PRP.'

(16) The Graham-Little syndrome Follicular keratosis with cicatricial alopecia

DR RAJ KUBBA AND DR ARTHUR ROOK

Addenbrooke's Hospital, Cambridge

Mrs I.S., aged 46 years. Housewife.

History. This patient first noticed a small patch of alopecia in the frontal area of her scalp in 1970. This has persisted and many more patches have since appeared without any sign of regrowth. Since early 1974 she has gradually developed an irritable rash on her arms and back. She has been depressed for the past year or two and attributes this in part to losing her hair and the fear of impending baldness.

Past History. She suffered from meningitis as a child. She has no children.

Examination. In the scalp there were many small patches of cicatricial alopecia, some of which showed slight erythema. Some follicles showed plugging, but there was no evident folliculitis. On the extensor aspects of the forearms and in the lumbosacral region there were well defined large groups of horny follicular papules.

Histology. (1) Biopsy from the scalp. This showed destruction of hair follicles, follicular plugging and hyperkeratosis and an invasive perifollicular infiltrate consisting of lymphocytes with a few histiocytes.

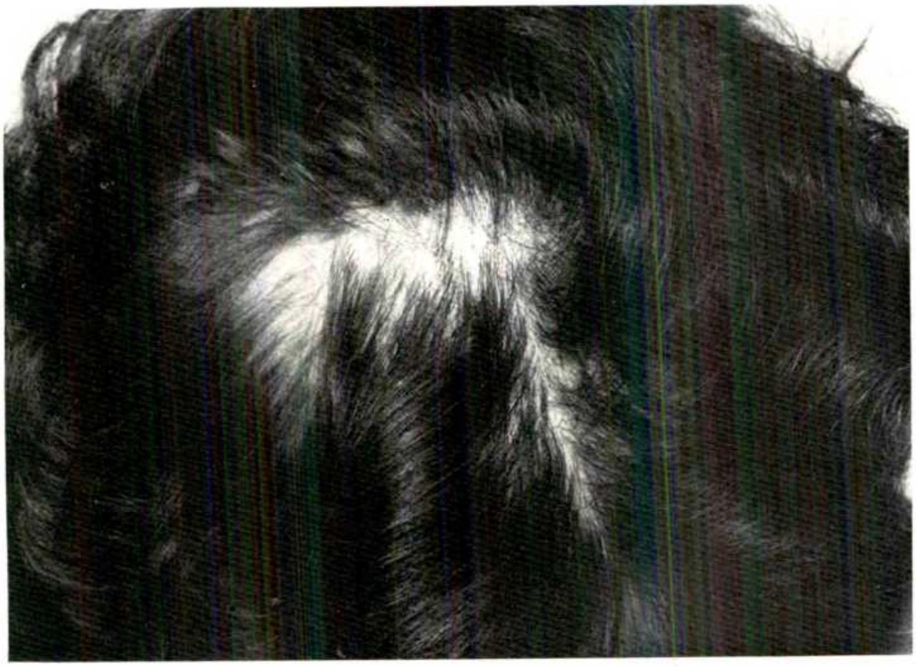

FIGURE 1. Cicatricial alopecia.

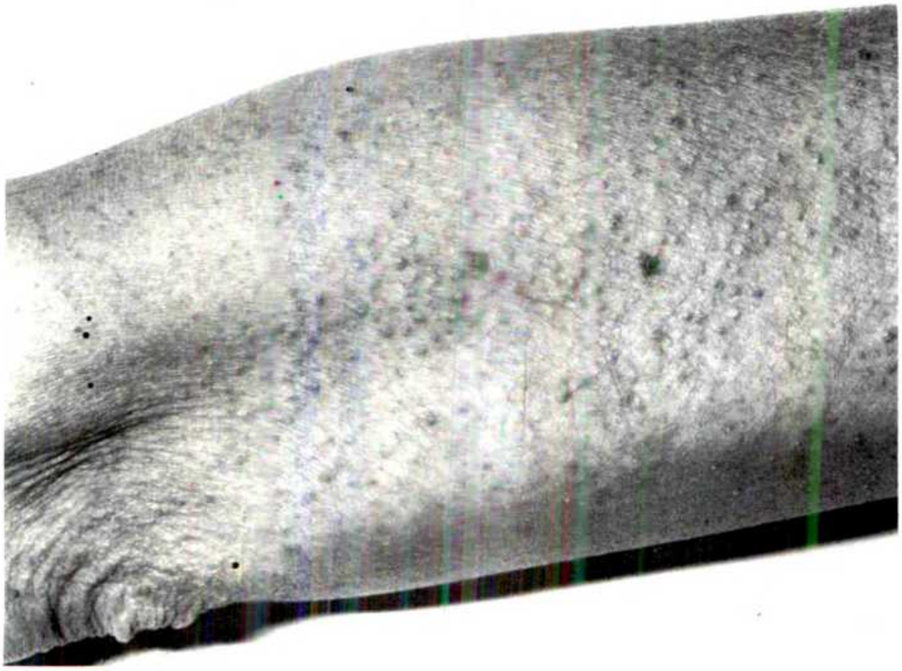

FIGURE 2. Follicular lesions on arm.

(2) Biopsy from the forearm. This showed similar but more severe changes than the scalp biopsy. In one area a granulomatous infiltrate was seen surrounding a ruptured follicle.

Management. The skin lesions have cleared with local applications of retinoic acid (Retin A). Her scarring alopecia is being treated with intralesional steroids through a Porton jet injector, but so far no improvement is evident.

Discussion. This is the so-called Graham–Little syndrome, which is also associated eponymously with Lassueur of Switzerland and Piccardi of Italy. The essential clinical features of the syndrome are cicatricial alopecia and follicular keratosis: the horny papules are usually grouped in plaques but may be widely disseminated. Most of the patients have been women aged 30–60 years. The time relations of these two manifestations of the syndrome are interestingly variable. Both may appear more or less simultaneously, but more often the alopecia precedes by months or years (e.g. 10 years) (Graham-Little, 1915; Beatty & Speares, 1915) the sudden onset of follicular papules on the trunk and limbs. The papules may disappear after a few months only to recur after an interval (Senear, 1920) whilst the alopecia progresses slowly but relentlessly. In some cases pubic and axillary hair is lost, without clinical evidence of scarring in these sites, although histologically the follicles are found to have been destroyed.

Much inconclusive discussion has been concerned with whether or not this syndrome is a variant of lichen planus (Pagès *et al.*, 1961). The most recent studies (Alessi & Dal Pozzo 1968; Valentino *et al.*, 1974) favour the view that it is a distinct entity.

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(17) Keratosis pilaris with recurrent folliculitis decalvans

DR RAJ KUBBA, GROUP CAPTAIN J.N.S.MITCHELL AND DR ARTHUR ROOK

Addenbrooke's Hospital, Cambridge

Mr P.M., aged 18 years. Hospital case no. 44 92 80.

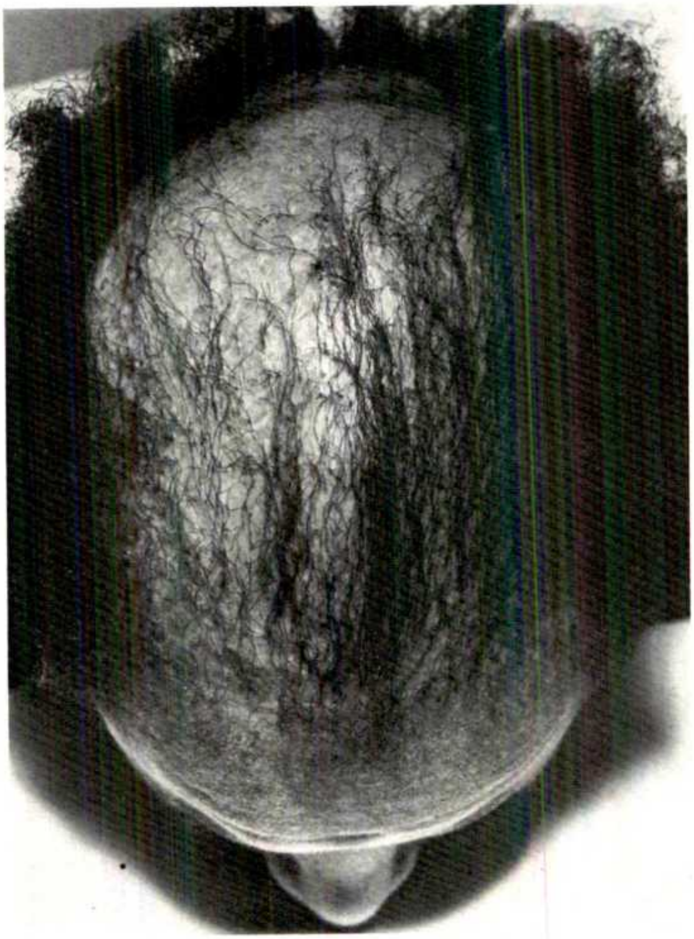

FIGURE 1. Cicatricial alopecia.

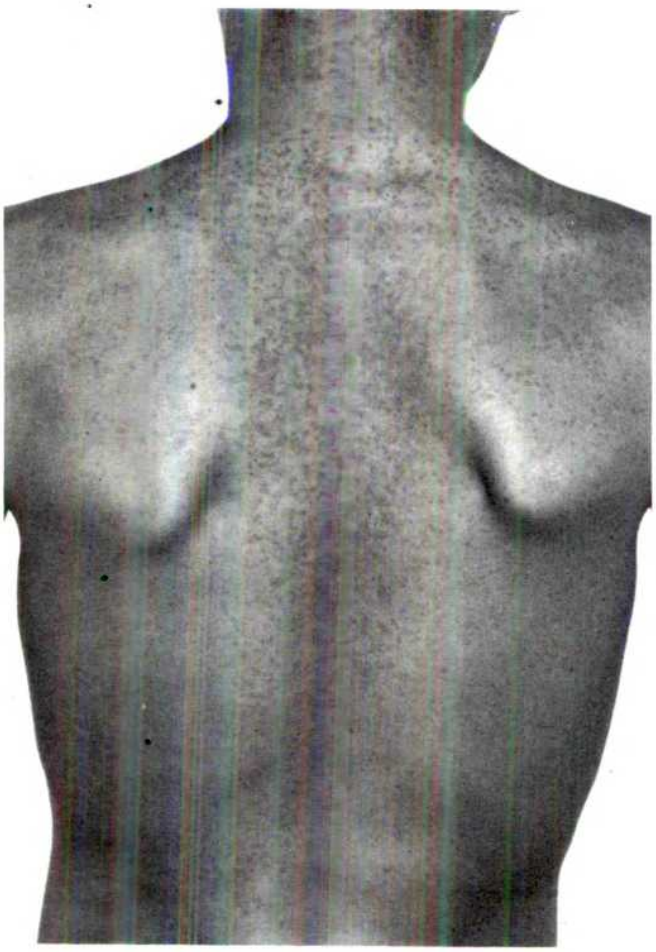

FIGURE 2. Follicular lesions on back.

History. This patient was born prematurely and when 5 days old underwent an abdominal operation for some reason now unknown. His head was noted to be scurfy at birth and his skin has always been dry and rough. His main problem however has been repeated infections of the scalp from the age of 2 years. These were frequently associated with severe irritation and scratching. He started to lose hair when aged 5 years and this continued until 3 years ago.

Family history. His parents are unrelated and no similar condition is known to have occurred in the family.

Examination. He is racially part Caucasoid and part negroid and shows normal physical and mental development. There is marked diffuse, cicatricial alopecia of the fronto-vertical areas of the scalp with normal negroid hair in other areas. His cutaneous eruption consists of horny follicular papules most profuse on the chest, the back and the extensor surfaces of the limbs. His nails and teeth are unaffected.

Histology. A biopsy of a papule from the back showed features of keratosis pilaris. There was no dyskeratosis or any other features of Darier's disease. Another biopsy of a papule from the back of the neck showed atrophied hair follicles surrounded by foreign body giant cells, acanthosis of the overlying epidermis, and a moderate perivascular infiltrate.

Treatment. He has received intermittent antibiotics, systemically as well as topically, for recurrent scald infections. About 3 years ago, however, he was started on continuous oral tetracycline (500 mg daily), which has prevented further hair loss; in fact there is some evidence of regrowth in the alopecic areas. He is now beginning to grow a beard and this so far is unaffected. His cutaneous eruption has never been troublesome enough to require treatment.

Discussion. There are several syndromes combining keratosis pilaris and cicatricial alopecia which are proved to be hereditary, and others which, from their early onset, are believed to be so. Cockayne (1933) accepted as 'ichthyosis follicularis with alopecia' a rare syndrome reported only in boys and showing many of the features present in our patient. There was insufficient evidence to determine the mode of inheritance. Zeligman & Fleisher (1959) described under the same label two negro boys aged 8 and 3 years with complete or almost complete absence of hair and with widespread keratotic papules.

Kuokkanen (1971) reported in five members of a family a syndrome which appeared to reach its full development only in males. Keratosis pilaris decalvans of the face and later of the neck and limbs was associated with redness and scaling of the scalp leading to hair loss, and with keratoderma of the distal third of the palms.

Degos & Delzant (1961) drew attention to the association of keratosis pilaris rubra with cicatricial alopecia of early onset.

The recurrent episodes of infection in our patient are not mentioned as such in the case reports we have studied, but 'inflammatory changes' are referred to both by Cockayne (1933) and by Kuokkanen (1971).

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(18) Xanthoma tuberosum and gout

DR J.B.LYON

Ipswich Hospital

R.P., male, aged 49 years. Electrical Salesman. Hospital case no. 36 36 58.

History. He has had lumps on his elbows, thighs and heels for 15 years. Recently he has had breathlessness on exertion. Apart from gaining weight, and occasional bronchitis, he has otherwise kept well over the past 20 years.

Family history. Non-relevant.

Previous history. He had infective hepatitis in 1942.

Examination. There are typical lesions of xanthoma tuberosum on his elbows, buttocks, thighs, knees and Achilles tendons. There are also gouty tophi on his ears. He is over weight. His blood pressure was 190/120 mgHg, but with treatment it is now 140/100 mmHg, and he is no longer in cardiac failure.

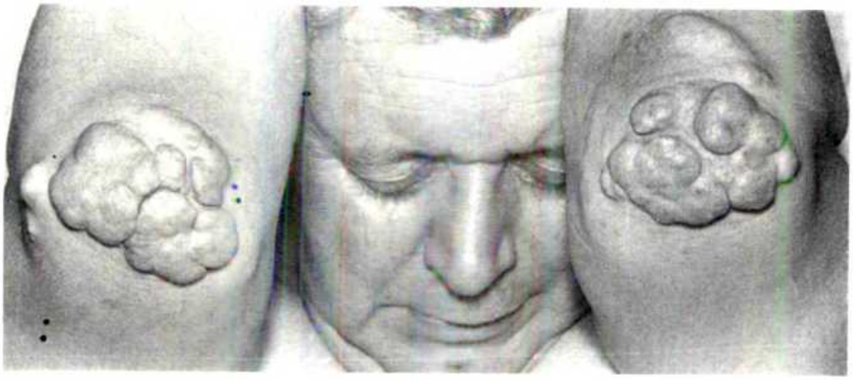

FIGURE 1. Tuberous xanthomata over elbows.

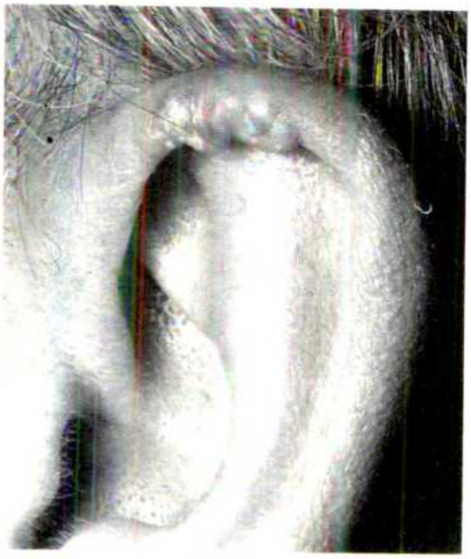

FIGURE 2. Gouty tophus on ear.

Investigations. Histology of nodules from his buttock and elbow confirms the diagnosis of xanthoma with typical foam cells, fibrous tissue, and no inflammatory reaction. Glucose tolerance test was normal. Fasting blood cholesterol 190 mg%; triglycerides 135 mg%; beta-lipoproteins 368 mg%; prebetalipoprotein 116 mg%; chylomicrons 6 mg% (all normal). His uric acid was 9·4 mg%, falling to 5·0 mg% with treatment. His blood urea was 82 mg%, falling to 43 mg%, and his LDH 460 units falling to 270. ECG showed left ventricular hypertrophy. Chest X-ray showed pulmonary oedema.

Treatment. He has been treated with Lanoxin, frusemide, Slow K, propranolol, methyl dopa, allopurinol and weight reduction.

(19) Lipid deposition in the dermis following renal transplant

DR DAVID GOLDIN, DR S.O.B.ROBERTS AND DR D.B.EVANS

Addenbrooke's Hospital, Cambridge

J.B., female, aged 40 years. Hospital case no. 39 30 98

History. Following a renal transplant 2 years before, in October 1972 a rash developed on both arms and on her left thigh over a period of about 6 weeks. Since then there has been a tendency for the eruption to extend distally and possibly to clear at the proximal borders. There is no irritation. At the time of onset she was taking prednisolone 17·5 mg daily, azathioprine 100 mg daily, methyl dopa, frusemide and potassium supplements. She also has severe steroid-induced bilateral aseptic necrosis of both femoral heads and is awaiting an operation for bilateral hip replacement.

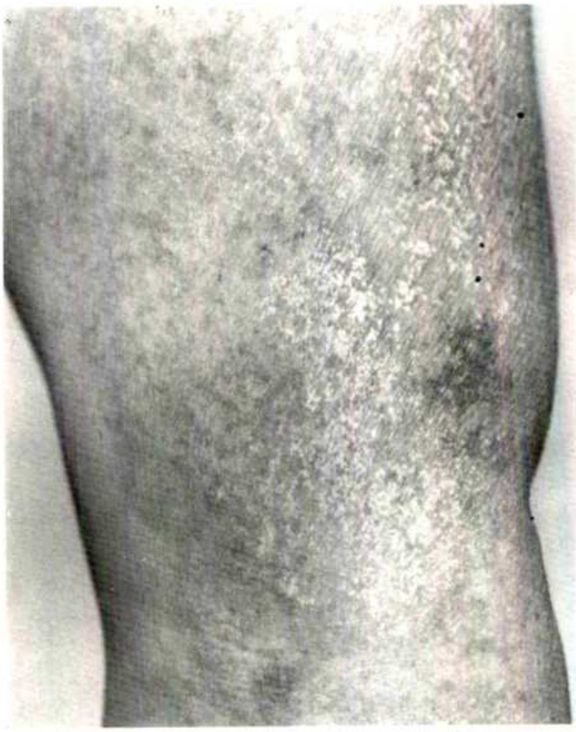

FIGURE 1. Lipid deposits on upper arm.

Past History. Prior to her present troubles, she never suffered from any skin disorders, nor was pruritus a problem when in renal failure. Proteinuria was initially detected in her first pregnancy in 1957. She suffered from pre-eclampsic toxaemia in three subsequent pregnancies in 1960, 1962 and 1965. Anti-hypertensive therapy was first commenced by her general practitioner in 1968 and at that time her blood urea was 68 mg%. By June 1972 she was considered to be in terminal renal failure due to bilateral pyelonephritis and in September of that year she had a bilateral nephrectomy and was started on the intermittent haemodialysis programme. Transplantation took place in October

1972 and her subsequent progress with regard to her renal function, despite two early episodes of threatened rejection, is considered satisfactory.

Examination (December 1974). Her skin is generally thin and atrophic, presumably due to the steroid therapy. On the antero-medial aspects of the arms and the antero-lateral aspect of the right thigh there are clearly defined sheets of abnormal skin. The central area of a typical eruption has a reticulate pattern with small papules, some creamy and others more yellow coloured on a dusky background. The appearance is rather similar to pseudoxanthoma elasticum. Around these areas are margins of erythematous skin about 2 cm wide.

Investigations. Biopsies were taken both from an active margin of a lesion and also from the centre, but rather surprisingly the histopathology of both was identical: 'The epidermis is thin and mildly atrophic but shows no other significant abnormality. The dermis shows separation and fragmentation of collagen bundles and elastic fibres and an excess of mucinous material between the fibres. In the papillary dermis there are large empty spaces, some surrounded by multinucleate giant cells. Frozen section reveals that these spaces are filled with lipid and that, although concentrated in the upper dermis, these deposits are present between the collagen bundles in the reticular dermis. Scattered macrophages and giant cells contain vacuoles of fat, but a large amount is extracellular. Foamy histiocytes are not a feature and there is no chronic inflammatory response. The properties of this lipid material (it is doubly refractile and is seen as a fine basketweave pattern which disappears on heating, it is completely dissolved in paraffin sections, it is stained by Sudan dyes, has slight PAS positivity), indicate that it is a mixture of substances, predominantly neutral fat and triglycerides. It does not exhibit the properties of cholesterol. The distribution of the lipid in the dermis indicates that it is due to lipid deposition rather than to alteration in composition of pre-existing fat. Thus these lesions are eruptive xanthomata of the type usually secondary to hyperlipaemia' (Dr J.R.Anderson).

Shortly after the onset of the rash, a fasting serum cholesterol was 372 mg/100 ml (normal range 140–240 mg/100 ml) and the serum triglycerides 340 mg/100 ml (normal range 50–150 mg/100 ml). The serum lipoprotein estimation showed a Fredrickson type IIb pattern. Overt diabetes has not been a problem.

Comment. This lady has developed eruptive xanthomata in an unusual distribution. We know of no other reports of similar findings and there has been no development of xanthomata in a series of more than 100 post renal transplant patients examined by one of us (Roberts). Hyperlipidaemia and hypercholesterolaemia are commonly a feature of chronic renal disease as well as being a characteristic of the nephrotic syndrome. In their recent review, Cramp, Moorhead & Wills (1975) discussed this finding and the various possible causative mechanisms. Continuing hyperlipidaemia in post-transplant patients is thought to be due to continuous corticosteroid therapy (Ghosh *et al.*, 1973; Casaretto *et al.*, 1974), though normal serum lipids may be found in younger stable post-transplant patients on alternate-day corticosteroid therapy (Beaumont *et al.*, 1975). That accelerated atherosclerotic vascular disease accounts for the majority of deaths in patients on long term maintenance haemodialysis has been widely reported and it is probable that there is an increased prevalence of cardiovascular disease in post-transplant patients (Lowrie *et al.*, 1973; Gurland *et al.*, 1973; Evans, D.B., unpublished). The distribution of the xanthomata in this patient is curious and prompts us to speculate that the lipids are accumulated at sites where steroid-induced collagen degeneration may be greatest. Further studies and lipid identification are planned.

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(20) Anaphylactoid purpura caused by tartrazine and benzoates

DR RAJ KUBBA AND DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge

B.A., female, aged 49 years. Boarding kennels proprietress.

History. She has suffered from bronchial asthma since early childhood. Prick tests on several occasions have not revealed any exogenous allergic factors. From September 1973 she has had a recurrent purpuric eruption on her legs. This was not preceded by a systemic illness or an infection. The lesions appear in crops and are sometimes associated with a tingling sensation in the affected skin. Individual spots take a few hours to a few days to clear, and there have been periods of remission of up to 4 weeks. Over the same period her joints have often been painful and swollen. Prior to the onset of the eruption she had been taking beclomethasone (inhaler), prednisolone (2·5 mg daily), salbutamol and silbephylline.

Examination. This only showed about 20 small inflammatory purpuric lesions up to 2 mm across scattered over her legs. There were also pulmonary signs of bronchial asthma.

Provocation tests. These were carried out with Tartrazine, Sunset yellow, Neococcine, sodium benzoate, methyl-4-hydroxybenzoate and aspirin. One substance was given each day with increasing doses every 2 hours. She gave positive provocation tests with the smallest doses used of Tartrazine (1 mg), sodium benzoate (50 mg) and methyl-4-hydroxybenzoate (50 mg). In each instance small erythematous macules were seen on the legs within 2 hours. They increased in number and became purpuric. A peak was reached between 6 and 8 hours after which they faded. During the provocation tests with benzoates she was wearing an elastic support bandage on one leg to facilitate healing of the skin biopsy wound; purpura did not appear under the bandaged skin. Her asthma was not changed during the provocation tests.

Histology of a lesion provoked by Tartrazine showed a leukocytoclastic vasculitis.

Management. She has now been on a diet free of Tartrazine and benzoates for nearly a year. During this period her purpura has recurred on a few occasions, but each time it could be related to eating something she should not have eaten. Treatment of her asthma has remained unchanged.

Comment. Criep (1971) reported the first case of recurrent purpura caused by Tartrazine. Michaëlsson, Pettersson & Juhlin (1974) reported a further seven cases of purpura caused by Azo dyes or food and drug additives. In some of their cases asthma was also made worse by the Tartrazine. However, this was not found in our patient.

Azo dyes and food additives are best known for their ability to aggravate urticaria (Michaëlsson & Juhlin, 1973). We also have under our care two patients with vasomotor rhinitis, who appear to have improved on a Tartrazine free diet.

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(21) Mycosis fungoides with necrotising angiitis

DR DAVID GOLDIN AND DR ARTHUR ROOK

Addenbrooke's Hospital, Cambridge

G. de B., male, aged 54 years. Accountant. Hospital case no. 16 49 47.

History. He first presented in 1959 when he was 38-years-old, complaining of a very painful ulcer of 14 months duration on the left buttock. This had healed once but quickly broke down again. There had been no obvious cause for it. For many years previously he had had a red, scaly, non-irritable rash on the thighs and forearms.

On examination there was an indolent ulcer with surrounding livedo suggesting a vascular pathogenesis. There were patchy erythema, scaling, atrophy and purpura on the arms and legs suggestive of a reticulosis. The histology of a biopsy taken from the left elbow was compatible with the diagnosis mycosis fungoides. However, successive histological studies of the ulcer showed vascular damage and a diagnosis of polyarteritis was suggested. In June 1960 the ulcer was successfully treated by wide excision and a Thiersch graft.

Over the next 5 years he remained reasonably well and did not attend the clinic. Then in 1965 he was seen again as an area of induration, ulcerated at two points, had developed on the left buttock. Over the next few years nodules developed at irregular intervals on the trunk and limbs. These nodules usually ulcerated but eventually healed on topical antibiotic therapy. Although these lesions were apparently inflammatory on clinical examination, they showed the histological changes of mycosis fungoides.

Poikiloderma became established on his limbs and, by 1966, the typical changes of parapsoriasis en plaque were seen. Pruritus was not a problem. He was given a course of β-ray therapy in 1973 with subsequent clearing of the cutaneous nodules which had become rather widespread.

In February 1975 he was admitted to hospital with an extensive, acute, apparently eczematous eruption which was thought to be secondary to an area of infected eczema on the right ankle. This cleared quite quickly when treated with cloxacillin by mouth and a topical corticosteroid cream. The histology of biopsies taken from the right ankle and the chest was that of an acute vasculitis. He had not been receiving any medication during the weeks preceding this episode.

Past History. When he was sixteen he developed a 'weeping dermatitis' which was cured when he joined the army 8 years later. In 1972 he suffered from hidradenitis of the right axilla which improved considerably following a course of clindamycin.

Examination. The changes of parapsoriasis en plaque are the most constant feature. The skin surrounding the graft on the left buttock shows some livedo. Physical examination is otherwise normal.

Investigations. Histology of a biopsy taken from the ulcer on the left buttock (1960): 'The epidermis is atrophic with areas of necrosis in it; some papillary hyperkeratosis and follicular plugging is seen. A band of inflammatory cells (lymphocytes, histiocytes and polymorphonuclear leukocytes) fills the upper corium and extends into the epidermis in places. The deep surface of the infiltrate is unusually sharply defined save where it extends into hair follicles. There are sparse clusters of infiltration also in the walls of subcutaneous vessels, which however do not appear to be thrombosed. The lesion is, I think, most likely of vascular origin and polyarteritis would be a probable cause' (Professor G.A. Gresham).

Further biopsies showed conspicuous perivascular infiltrates of chronic inflammatory cells and some organized intravascular thrombi.

Histology of skin taken from the left elbow (1960): 'The papillary layer of the corium is heavily infiltrated by cells, mainly histiocytes. The infiltration extends into the epidermis and is present around some blood vessels deeper in the corium. Some of the histiocytes appear abnormal and a few are in mitosis. There is some acanthosis and parakeratosis. No vascular lesions are seen. These findings strongly favour a diagnosis of mycosis fungoides. It is possible that, as the cellular infiltrates in the earlier specimens are not dissimilar, mycosis fungoides may well be the basic disorder.'

Histology of a biopsy of the recent apparently eczematous rash (1975): 'There are tiny parakeratotic scales, patchy epidermal spongiosis and moderate sub-epidermal oedema with areas of recent haemorrhage. A moderate infiltrate of polymorphonuclear neutrophil leukocytes and lymphocytes is present, around small vessels in the dermis, and there is conspicuous proliferation of capillaries. The appearance is that of a vasculitis.'

Blood (March 1975): Haemoglobin 16·5 grams/100 ml. White blood count 8000 cells/mm^3. Platelet count normal. ESR (Wintrobe) 1 mm/h. Serum protein 6·5 g/100 ml. Albumin 3·9 g/100 ml. Protein electrophoresis is normal. Urea, electrolytes and liver function tests are normal.

Chest X-ray is normal.

Comment. This case is unusual in several respects. During the 16 years that he has been under observation he has shown in varying combinations, tumours of mycosis fungoides, poikiloderma developing after the initial tumour, and episodes of necrotizing vasculitis.

It is interesting to speculate that the vasculitis and the mycosis fungoides may both be manifestations of antigen persistence (Tan *et al.*, 1974). The association of vasculitis with reticulosis has been reported. Two of Copeman's (1970) thirty consecutive patients with necrotizing vasculitis had Hodgkin's disease.

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(22) Pili torti

DR J.B.LYON

Ipswich Hospital

K.F., male, aged 4 years. Hospital case no. 33 92 37.

At birth his hair was abundant and fine. From the 4th month it was progressively replaced by sparse, coarse hair. Physical and mental development have been normal.

Family history. The parents are not related. Two siblings have normal hair.

Examination. The head hair is sparse, coarse and unkempt, somewhat resembling a wire brush, and with a spangled appearance. The eyebrows are reduced to short, irregular stumps. The lower eyelashes are sparse and irregular. The child's hearing appears to be normal.

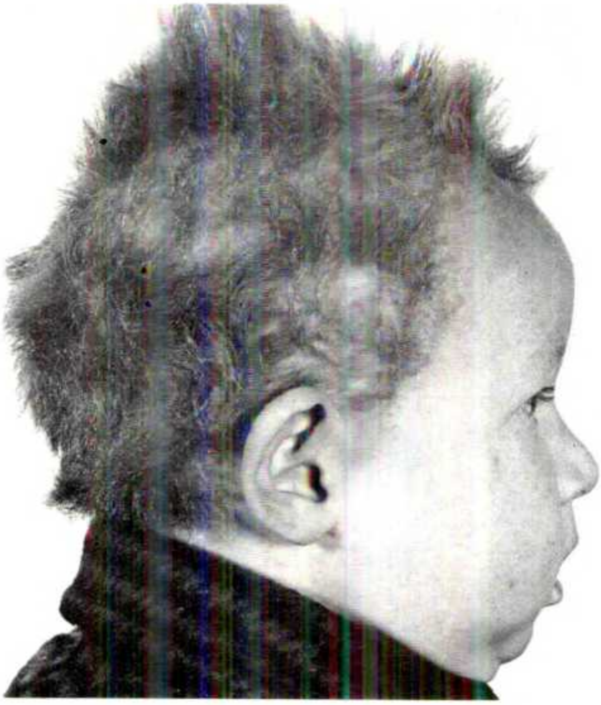

FIGURE 1. Pili torti.

Investigation. On microscopy the hairs show regular twisting of the shaft on their own axes. Scanning electronmicroscopy has been arranged.

Discussion. Most cases of pili torti as an isolated defect are clearly determined by an autosomal dominant gene or are sporadic.

Pili torti occurs also as a component of Björnstad's syndrome (Björnstad, 1941) when it is associated with sensorineural deafness. In Crondall's syndrome twisted hair is associated with deafness and hypogonadism.

REFERENCE

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(23) Facial hemiatrophy

DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge

R.Y., male, aged 13 years. Hospital case no. 44 00 21.

History. Five years ago he noticed that the left side of his upper lip chapped easily. Since then there has been progressive deformity of his lip and tongue. Ill-defined skin lesions have been present on his arms and body for a year or two, and are becoming more obvious.

Examination. There is marked soft tissue atrophy of the left side of his upper lip and the left half of his tongue, with less noticeable changes elsewhere on the left side of his face. His teeth show a small malocclusion in the upper left quadrant (canine and premolar).

On his arms and upper chest there are several atrophic areas up to 10 cm across resembling atrophic morphoea. These show no violaceous edge and no sclerosis.

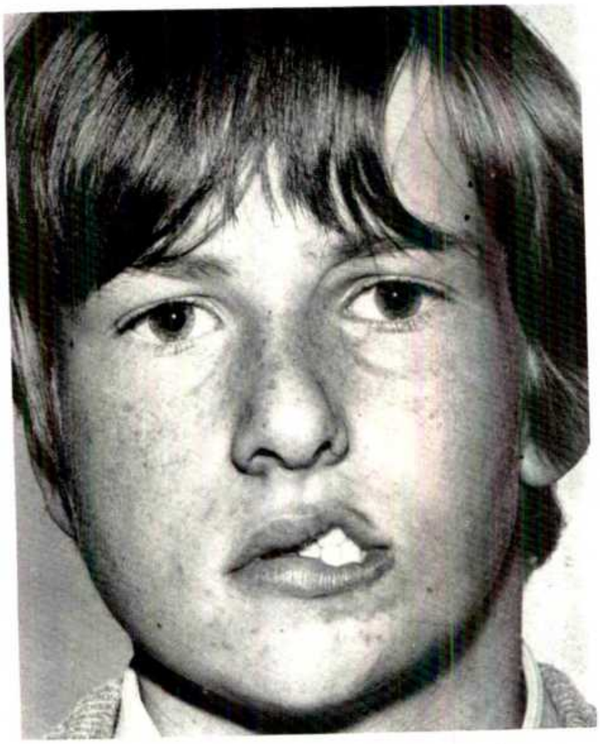

FIGURE 1. Facial hemiatrophy.

Investigations. X-rays of his skull and upper jaw, including an orthopantomogram, show no abnormality apart from the malocclusion. Dental models, however, do show definite evidence of bone loss in upper left canine and premolar region. The soft tissue abnormality has also been recorded, and will be followed, by stereophotogrammetry.

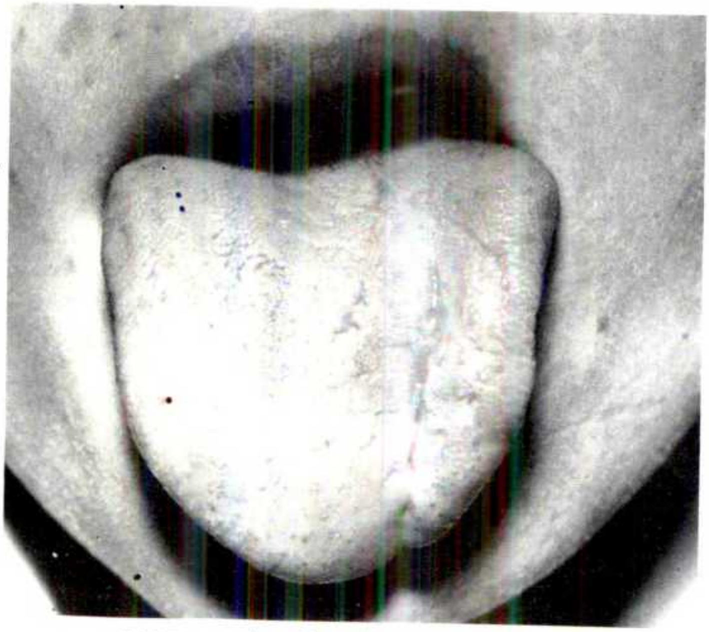

FIGURE 2. Atrophy of left half of tongue.

Comment. Treatment with penicillamine has been considered, but not given. An orthodontic opinion (Mr S.I.M.Robinson) is that, with so much loss of bone, straightening of the misplaced teeth is not feasible, and, even if successful, would not materially reduce the disfigurement.

There may be some place for plastic surgery eventually.

(24) Plasma cell gingivitis

DR DAVID GOLDIN, DR ARTHUR ROOK AND MR W.T.LAMB

Addenbrooke's Hospital, Cambridge

E.W., female, aged 46 years. Hospital case no. 44 09 19.

History. The patient began to complain of soreness of the gum above the upper incisors 4 years ago. The condition has remained static. It has not been helped by topical applications nor by correction of her iron deficiency anaemia.

Examination. The free and attached gingivae in the arc above and between the upper canines are reddened and oedematous, with a velvety surface. The boundary of the inflamed area is sharply demarcated from the rest of the gingivae, which appear normal.

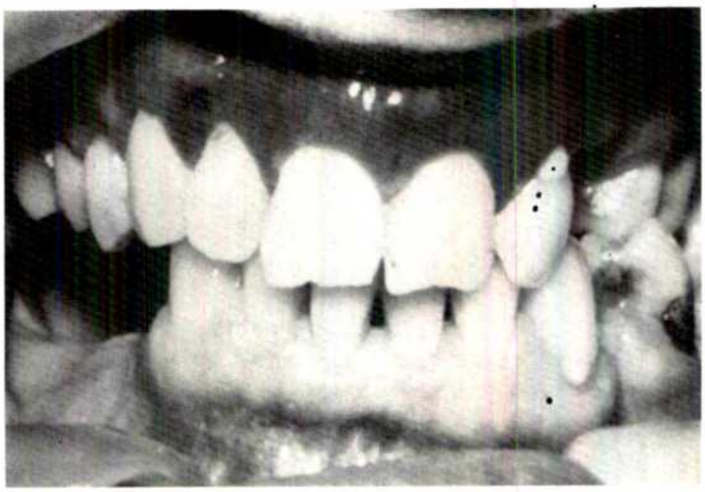

FIGURE 1. Plasma cell gingivitis.

Histology. A biopsy of the affected mucosa showed a severe, chronic inflammatory reaction of the sub-epithelial connective tissue, consisting of lymphocytes, plasma cells and occasional mononuclear cells. The reaction extended in places into the squamous epithelium.

Treatment. None recently.

Discussion. After Zoon (1952) had differentiated plasma cells balanitis from erythroplasia, similar chronic lesions, rich in plasma cells, were recognized on the vulva and on the lips, and this led Schuermann in 1958 to propose the term circumorificial plasmocytosis.

The condition has been observed in various sites in the oral mucosa and has been considered on questionable evidence to be an inflammatory response, characteristic of periorificial mucosa, to a variety of physical or chemical insults.

Plasma-cell gingivitis has rarely been reported. Duperrat *et al*. (1961) presented such a patient in whom plasma-cell proctitis was also found.

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(25) Relapsing polychondritis

DR R.H. CHAMPION AND DR ANNE NICHOLLS

West Suffolk Hospital, Bury St Edmunds

R.M., female, aged 33 years. Housewife.

History and examination. She presented in July 1974 with a few weeks history of a painful swelling in the well of her right ear. Histology confirmed the diagnosis of relapsing polychondritis. Soon afterwards she developed pain in her costal cartilages, in her left ear, and later in the bridge of her nose. She has also had pain and stiffness in her knees, shoulders and hands, and at times has had marked effusions in both knees. These pains have been of considerable severity. She has also felt slightly short of breath on exertion.

Treatment in hospital and at home with systemic corticosteroids (up to 60 mg daily of prednisone), azothioprin (150 mg daily for many weeks, up to 300 mg for a short time), indomethacin and other anti-inflammatory agents has barely controlled the worst of her symptoms. Recently she has been investigated and treated at the Charing Cross Hospital by Dr Maini. She has been no worse since leaving off the azothioprin, and reducing the prednisone to 9 mg daily.

Investigations. Blood count normal. ESR 3 mm/h. Serum proteins and electrophoresis normal. IgA 245 mg; IgG 236 mg; IgG 750 mg/100 ml. Latex test negative. No anti-DNA antibodies. No L.E. cells.

X-rays of joints and chest and *tomograms* of trachea are all normal.

Bronchoscopy and lung function tests normal.

Arthroscopy of her knee showed a few frond-like cartilaginous processes (Dr D.Yates).

Immunological studies. The results of investigations for circulating antibodies and delayed hypersensitivity to cartilage (Rajapaske & Bywaters, 1974) are awaited.

REFERENCE

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(26) Sarcoidosis

DR M.A.SMITH

Essex County Hospital, Colchester

Mr P.G., aged 52 years. Schoolmaster. Hospital case no. U.50 76 41.

History. For 3 years he has had a symptomless eruption on his trunk and arms. He is otherwise in good health.

Examination. He has skin lesions 1-3 cm across, irregular in shape, scattered on his trunk and arms. These are faint bluish in colour, smooth and flat. His spleen is palpable. Blood pressure 170/100 mmHg. Ophthalmological examination—normal.

Investigations. Skin biopsy. 'In the dermis there are several sarcoid bodies, some with giant cells. The appearances are compatible with sarcoidosis'.

Tuberculin Tine test negative. Chest X-ray—normal. Blood count and ESR—normal.

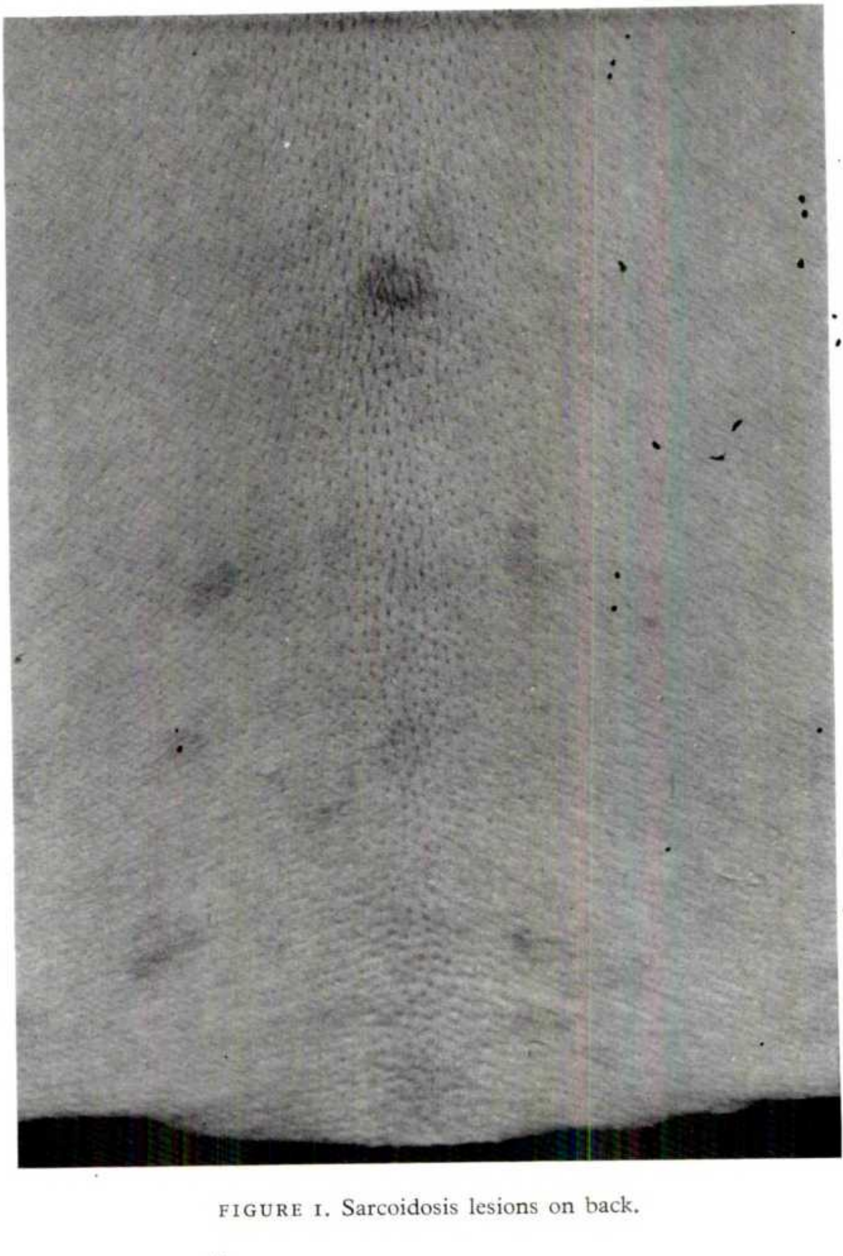

FIGURE I. Sarcoidosis lesions on back.

(27) Focal dermal hypoplasia

DR R.H.CHAMPION

Addenbrooke's Hospital, Cambridge

E.H., female, aged 11 years. Hospital case no. 39 19 32.

History. She presented in 1972 with a recurrent warty papilloma on her lip. A papilloma of her palate had been removed in 1971. There was also a history of 'eczema', which had been present probably from birth.

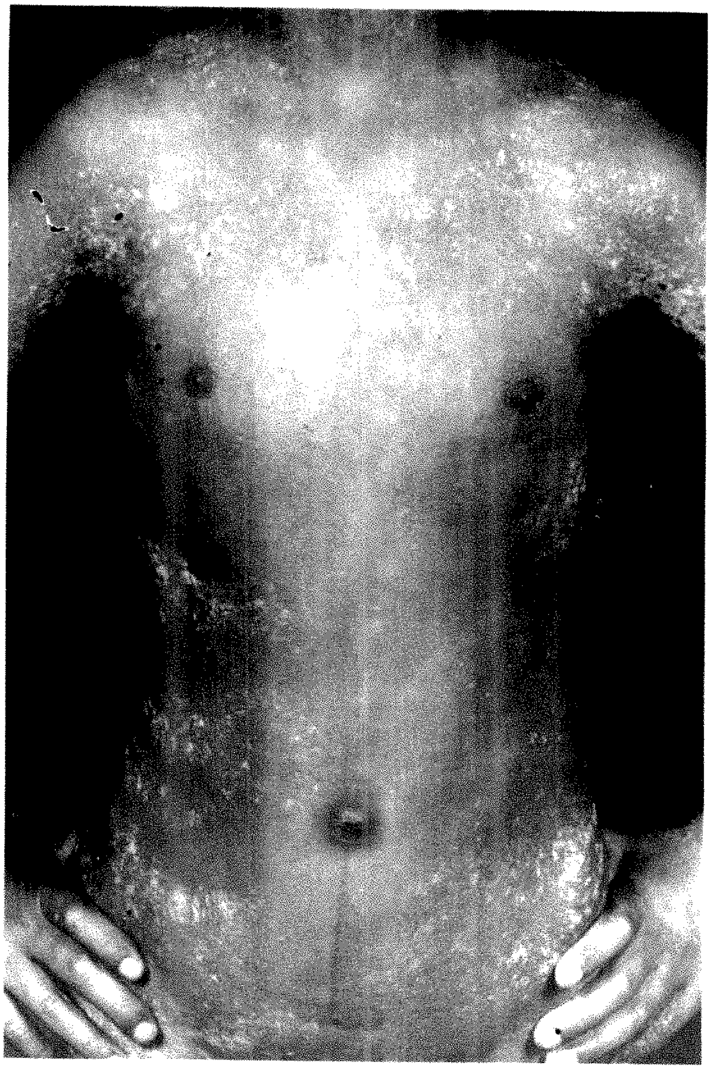

FIGURE 1. Focal dermal hypoplasia. (u.v. filter Chance O.X.1–3mm. Braun F 800 electronic flash. F.P.4 film f/11.)

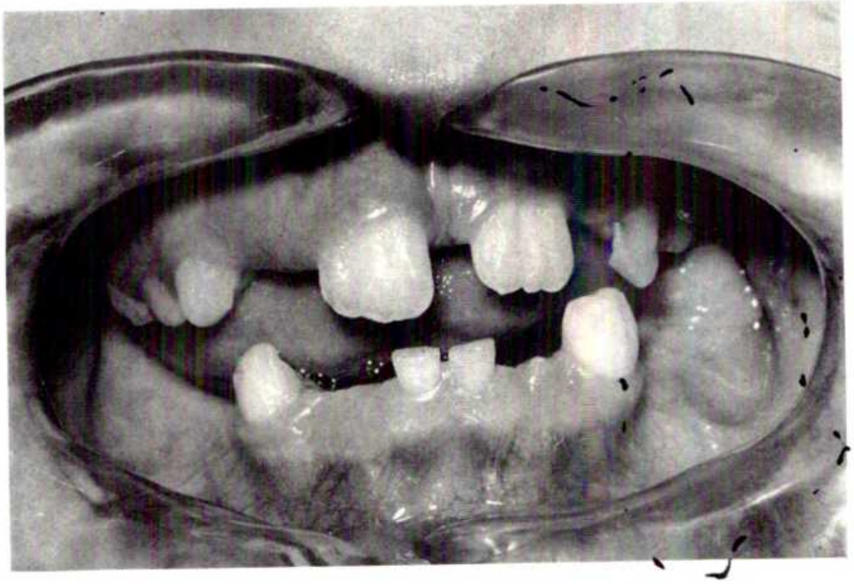

FIGURE 2. Unerupted and abnormal teeth.

Family history. Her mother has had a similar eruption on her arms from childhood, but has normal teeth. Her grandparents, three maternal aunts and three sisters are all unaffected.

Examination. Skin. There are stellate, atrophic, erythematous scar-like lesions on her limbs, body, lips and scalp. Her nails are atrophic and show longitudinal ridging.

Teeth. She has absent, unerupted and poorly formed teeth, with gnarled atypical enamel.

Investigations. X-rays of the long bones show evidence of osteopathia striata (as described by Howell & Reynolds, 1974).

REFERENCE

HOWELL, J.B. & REYNOLDS, J. (1974) Osteopathia striata—a diagnostic osseous marker of focal dermal hypoplasia. *Transactions of the St John's Hospital Dermatological Society*, **60**, 1[illegible]8.

(28) Epithelioid sarcoma of the pulp of the left ring finger (Demonstration)

DR J.B.LYON

Ipswich Hospital

M.T., male, aged 33 years. Machinist. Hospital case no. 17 51 [illegible]7.

History. At the age of 14 years a wart on his left ring finger pulp was treated by curettage and diathermy. The area remained well healed until 5 years ago when a firm nodule appeared. In the previous year this had become ulcerated.

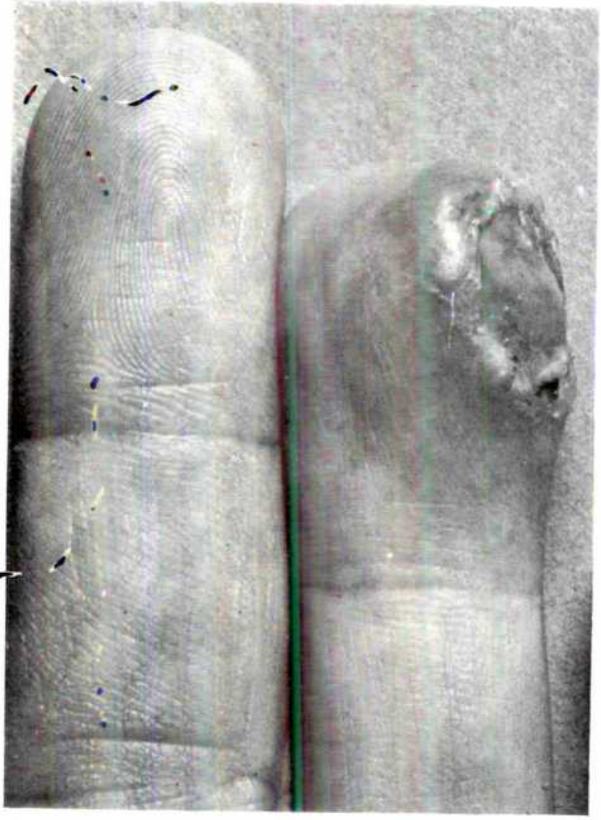

FIGURE 1. Ulcerated lesion on finger tip.

Examination. There was an area of ulceration 1·2 cm across on the pulp of the left finger with an infiltrated rolled edge, suggesting a squamous epithelioma. There was no lymphadenopathy.

Treatment. Amputation at the proximal interphalangeal joint was performed in September 1973. A 2-year follow-up shows no signs of metastases.

Investigations. X-rays of the fingers showed no bony involvement.

Histology. There was a cellular tumour spreading diffusely in the dermis and composed of fairly large rounded or polygonal cells, mostly in lobulated masses. There were fairly numerous mitoses. There was some increase in recticulin. Professor G.A. Gresham suggested the diagnosis of Epithelioid Sarcoma.

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Acknowledgements

We are extremely grateful to Professor G.A.Gresham and his department for help with the histopathology, and to the Medical Illustration Service of Addenbrooke's Hospital for help with the illustrations.

(Continued from back cover)

Nickel Sensitivity Resembling Bullous Pemphigoid; R. KUBBA AND R. H. CHAMPION 41
Morphoea Following Chicken Pox; J. SINGH AND G. A. BECK 43
Morphoea Arising in a Hypoplastic Breast; D. GOLDIN AND R. H. CHAMPION 44
Calcinosis Universalis; R. KUBBA AND R. H. CHAMPION 45
Urticaria, Amyloidosis and Deafness. Muckle Wells Syndrome; R. H. CHAMPION 46
Follicular Keratoses; D. GOLDIN AND R. H. CHAMPION 48
Folliculitis; J. SINGH AND G. A. BECK 49
Follicular Pityriasis Alba; G. A. BECK 51
The Graham-Little Syndrome: Follicular Keratosis with Cicatricial Alopecia; R. KUBBA AND A. J. ROOK 53
Keratosis Pilaris with Recurrent Folliculitis Decalvans; R. KUBBA, J. N. S. MITCHELL AND A. J. ROOK 55
Xanthoma Tuberosum and Gout; J. B. LYON 57
Lipid Deposition in the Dermis Following Renal Transplant; D. GOLDIN, S. O. B. ROBERTS AND D. B. EVANS 59
Anaphylactoid Purpura Caused by Tartrazine and Benzoates; R. KUBBA AND R. H. CHAMPION 61
Mycosis Fungoides with Necrotising Angiitis; D. GOLDIN AND A. J. ROOK 62
Pili Torti; J. B. LYON 64
Facial Hemiatrophy; R. H. CHAMPION 65
Plasma Cell Gingivitis; D. GOLDIN, A. J. ROOK AND W. T. LAMB 66
Relapsing polychondritis; R. H. CHAMPION AND ANNE NICHOLLS 67
Sarcoidosis; M. A. SMITH 68
Focal Dermal Hypoplasia; R. H. CHAMPION 70
Epithelioid Sarcoma of the Pulp of the Left Ring Finger (Demonstration); J. B. LYON 71

British Journal of Dermatology, Volume 93, Supplement 11, July 1975

CONTENTS

PAGE

Foreword 1
Programme of the meeting 4

Summaries of papers

Ochronosis and Colloid Milium from Hydroquinone in Man: 50 Cases, with Pathology and Electron Microscopy; G. H. FINDLAY 9
The Treatment of Hypermelanosis with 4-isopropylcatechol; S. S. BLEEHEN 9
A Controlled Study of Vitamin E Treatment in Epidermolysis Bullosa; R. H. ADAMS, R. A. MAIN AND R. A. MARSDEN 10
Acne and Eczema: a Familial Study; K. LIDDELL 10
Rosacea, Migraine, Dixarit; W. J. CUNLIFFE, S. G. TAN AND E. TEBBS 11
A Follow-up of Tetracycline Treated Rosacea (with Special Reference to Rosacea Keratitis); A. KNIGHT AND C. F. H. VICKERS 12
Pemphigus Induced by *D. penicillamine* an 'Experimental Pemphigus'; J. HEWITT, M. BENVENISTE AND M. LESSANA-LEIBOWITCH 12
Eosinophilic Spongiosis, a Clinical, Histological and Immunofluorescent Correlation; A. G. KNIGHT, T. J. DELANEY AND M. M. BLACK 13
Personal Experience with Clofazimine in the Treatment of Leprosy; R. D. AZULAY 13
Cutaneous [illegible]ts of Mongolism: a Clinical Study; F. W. GRANT AND D. I. MCCALLUM 14
Immun[illegible]es Simplex; R. W. GANGE, C. H. P. BRADSTREET, A. DE BATS, J. R. PARK AND E. L. RHODES 15
Are Moles just [illegible] Cells? C. B. BENTLEY-PHILLIPS AND R. MARKS 15
Naevus Spilus: a Light and Electron Microscopical Study; R. A. J. EADY AND T. C. COWEN 16
Identification of Lymphoid Cells in the Cutaneous Infiltrate of Skin Reticuloses and Lichen Planus; R. [illegible]-H. TAN, J. P. HAYES AND [illegible] BYF[illegible]M 16
International Mortality from Bullous Diseases since 1950; J. A. SAVIN 17
Immunopathological Findings in Herpes Gestationis; J. A. CARRUTHERS AND M. M. BLACK 17
Lichen Planus: Investigations of Carbohydrate Metabolism and the HLA System; N. J. LOWE AND A. G. CUDWORTH, INTRODUCED BY C. F. H. VICKERS 18
Challenge Test Battery in Chronic Urticaria; R. P. WARIN AND RUTH J. SMITH 19
Trichotillomania: a Pathogenic Interpretation of Histologic Changes in the Pilosebaceous Unit; J. M. LACHAPELLE, G. E. PIERARD AND F. FIERENS 21
A Study of Persistent Light Reaction in Quindoxin and Quinine Photosensitivity; B. E. JOHNSON, S. ZAYNOUN, J. M. GARDINER AND W. FRAIN-BELL 21
A Preliminary Report of a Vascular Abnormality Occurring in Men Engaged in the Manufacture of Polyvinyl Chloride; ANNE E. WALKER 22
The Periodontal Membrane in Systemic Sclerosis; N. R. ROWELL AND F. E. HOPPER 23
Case Report: Fingernail Growth in Peripheral Pustulosis; R. P. R. DAWBER AND J. P. N. BYRNE 24
Reactivation of Herpes Virus Hominis (HVH) Infection in Mice Treated with Cyclophosphamide and Prednisolone Sodium Phosphate (Codelsol); T. ROBINSON AND JANICE HIRD 24
Fluctuations in the Blood Flow of Forearm Skin; N. T. S. EVANS, P. F. D. NAYLOR AND B. MCA. SAYERS 25
The Bacteriology of Skin Cysts; W. C. NOBLE, BARBARA LEPPARD AND J. THOMSON 25
Lipase, Hyaluronidase and Protease Production from Bacteria Isolated from Acne Patients; C. D. ROBERTS, K. T. HOLLAND AND W. J. CUNLIFFE 26
Keratin Hydration and Sebum Excretion Rate; W. J. CUNLIFFE, W. D. H. PERERA, MARGARET WILLIAMS, SUSAN WILLIAMS AND G. TAN 27
An Animal Model for Screening Topical and Systemic Drugs for Potential Use in the Treatment of Psoriasis; [illegible] DUVIVIER AND R. B. STOUGHTON 27
Increased DNA and Protein Synthesis in the Involved and Uninvolved Skin of Patients with Psoriasis; [illegible] MAR[illegible] 28
Epidermal Structure and Metabolic Activity in Acromegaly; P. HOLT AND R. MARKS 29

Case Reports

Granuloma Annulare in Mauriac's Syndrome; D. GOLDIN, A. J. ROOK AND D. GAIRDNER 31
Pyoderma Gangrenosum; D. GOLDIN AND A. J. ROOK 31
Lichen Myxoedematosus, Crohn's Disease and Mucinous Ovarian Carcinoma; R. KUBBA, N. I. MCNEIL AND A. J. ROOK 34
Scleromyxoedema; R. KUBBA AND R. H. CHAMPION 36
Discoid Lupus Erythematosus; M. A. SMITH 38
Juvenile Generalized Pustular Psoriasis; J. B. LYON 40
Nummular Eczema with Staphylococcal Sensitivity; E. WELBOURN, R. H. CHAMPION AND W. E. PARISH 4[illegible]

(*Contents continued on inside back cover*)